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AGRICULTURAL METEOROLOGY: MONTHLY SEQUENCE OF SUMMER PRECIPITATION AT WINNIPEG, SWIFT CURRENT, AND EDMONTON[1]

BY J. W. HOPKINS[2]

Abstract

An analysis of the monthly sequence of rainfall during the summer period April-September of the years 1890–1937, made by expressing each annual sequence as a fifth-degree polynomial function of time, indicates that despite marked annual variability the average monthly precipitation at all three stations exhibits a definite seasonal trend, with the maximum incidence of rain in June or July. At Winnipeg, the relative monthly distribution remains, on the average, essentially the same in both wet and dry years, but at Swift Current and Edmonton it is modified to some extent, the midseason maximum being relatively more pronounced in seasons of above-average total precipitation. At Swift Current, both the total amount and one of the coefficients specifying the monthly distribution of precipitation show some oscillatory variation with time; and at Edmonton, there has been a slight progressive change such that a smaller proportion than formerly of the total precipitation now falls in the second half of the season (July-September). No consistent increase or decrease in rainfall over the 48-year period has been recorded at any of the three stations.

Introduction

In semi-arid regions, crop production may be markedly affected by the distribution as well as by the total amount of rainfall during the growing season. As has been pointed out by Barnes (1), it is a fortunate circumstance that in the Prairie Provinces of Canada this distribution is, on the average, favourable, the major part of the spring and summer precipitation in this region falling during the months of May, June, and July, when cereal plant growth is most rapid and most in need of moisture.

It is already well known (8) that the total amount of rain falling at any point in this region is subject to large annual fluctuations, partly of an irregular and at present unpredictable nature, but also showing some element of secular trend, owing to the alternation of periods that are, on the whole, of above- and below-average precipitation, although these are too inconstant as to both phase and amplitude to be reducible to any simple cycle of recurrence. The accompanying variations in the seasonal distribution of the total precipitation received have not, however, as yet been studied in any detail, although such

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investigations as have been made in other countries suggest that these variations may be a feature of considerable significance. Thus Fisher (3), by the introduction of novel methods, was able to demonstrate from the records of rainfall at Rothamsted, England, for the period 1854–1918, a progressive change in the sequence of precipitation throughout the year, of which the main feature was an increase in December rain, with perhaps some relative reduction in spring and autumn. Likewise Cornish (2) applying the same procedure to the records of precipitation at Adelaide, South Australia, found that whilst there was no statistically significant change in the annual totals over the 95-year period, 1839–1933, there was a definite oscillation, with a period of approximately 23 years and an amplitude of 30 days, in the incidence and duration of the important winter rains. Succeeding portions of the present paper accordingly examine some statistics bearing upon this aspect of the climate of the Prairie Provinces.

Data and Method

The data used were the monthly totals of precipitation, expressed as inches of rain, for the period April to September inclusive, in each of the years 1890 to 1937, recorded by the Meteorological Service of Canada (9) at three representative stations. These were Winnipeg, Manitoba (lat. 49° 53′ N., long. 97° 7′ W., alt. 760 ft.); Swift Current, Saskatchewan (50° 20′ N., 107° 45′ W., 2440 ft.); and Edmonton, Alberta (53° 33′ N., 113° 30′ W., 2158 ft.).

For quantitative examination of the variations in seasonal distribution, the six monthly totals for each year at each station were first reduced to hundredths of an inch per day, in order to allow for differences in the number of days per month. Then the resulting series of six values was expressed, using Fisher and Yates's table of multipliers (5, Table XXIII), as a polynomial function of time of the fifth degree. The analytic advantage of this is that the individual monthly values are replaced by six polynomial coefficients a', b', . . . f', each of which provides a measure of a different component of the sequence as a whole; thus, b' is indicative of any linear increase or decrease in the amounts of rain recorded from the first to the sixth month, c' of the tendency of the sequence to a central maximum or minimum, d' of non-linear asymmetry, and so on. Furthermore, as the terms of different degree ξ_0', ξ_1', . . . ξ_5' are mutually orthogonal, the variation of the coefficient of each may be examined independently of that of the others.

Analysis of Rainfall Sequence

Tables I, II, and III list the distribution coefficients computed for each year and station.

Features of Average Sequence

All of the six coefficients a' . . . f' in each table have a wide range of fluctuation, and, with the exception of a', which is necessarily positive, exhibit numerous alternations in sign. However, the t ratios (4) shown at the foot

TABLE I

SEASONAL RAINFALL COEFFICIENTS, WINNIPEG

Year	a'	b'	c'	d'	e'	f'
1890	8.8	60.2	−43.7	−55.3	24.3	84.7
1891	8.1	37.6	−46.9	−14.9	7.7	−131.5
1892	7.4	1.3	−24.3	−107.7	−5.7	24.9
1893	8.7	−29.8	−84.2	−29.8	34.4	52.0
1894	5.6	−27.2	51.6	− 3.2	26.0	−67.6
1895	6.6	−15.0	−59.1	58.5	−3.7	74.7
1896	11.2	−103.4	25.1	49.1	−0.3	−32.3
1897	6.3	−7.5	−86.2	−37.0	29.8	104.2
1898	7.9	22.4	−55.8	55.4	34.2	−161.0
1899	7.8	−15.3	−41.5	−24.3	−6.9	−83.7
1900	7.8	106.6	−13.9	−41.9	17.1	25.1
1901	11.6	20.9	−86.0	95.4	86.4	−250.2
1902	7.1	−24.2	−23.2	106.8	−3.8	−22.2
1903	7.3	19.7	−12.1	63.7	−30.7	131.9
1904	8.5	26.3	−99.9	12.3	39.1	45.3
1905	8.5	1.7	−102.9	71.7	18.9	21.9
1906	9.4	−28.5	−89.0	75.0	32.6	−75.0
1907	6.5	31.2	−59.3	−102.3	−5.7	28.5
1908	7.6	−7.6	−21.5	33.9	−8.5	−37.5
1909	7.4	24.7	−52.8	−124.8	−15.6	13.2
1910	6.2	20.5	16.8	31.0	−1.4	−56.8
1911	10.3	−39.9	−13.0	81.6	−33.4	84.0
1912	10.9	51.8	21.3	31.3	20.5	209.3
1913	6.7	50.3	−59.3	−63.7	−9.9	−106.7
1914	8.3	47.5	−73.0	−56.0	30.2	179.6
1915	6.4	50.3	42.9	85.3	40.5	−3.1
1916	7.7	23.3	−68.2	49.8	6.8	−37.2
1917	5.1	48.1	−40.5	−22.9	25.5	12.7
1918	6.4	5.5	−56.0	−41.0	5.6	−101.8
1919	10.0	42.9	−60.0	23.4	23.0	−54.0
1920	6.1	43.5	−5.1	81.5	8.5	−80.9
1921	8.2	42.9	9.1	−13.1	11.9	62.9
1922	8.0	37.6	−48.8	36.6	20.2	112.8
1923	5.5	−5.7	−45.9	22.3	7.9	97.7
1924	6.8	7.1	49.7	−52.9	31.5	40.9
1925	5.3	41.2	7.9	−16.3	−0.1	−98.3
1926	7.4	77.8	−21.3	27.3	10.3	−79.5
1927	8.5	−18.3	19.8	53.2	−37.2	7.4
1928	8.9	−8.3	−95.3	−16.3	−4.5	24.1
1929	5.1	−0.8	16.1	73.7	−3.5	27.7
1930	8.0	−9.8	−87.1	50.7	7.5	136.5
1931	6.7	50.7	−9.6	33.2	−3.6	77.2
1932	5.8	19.2	−28.2	3.2	23.2	−27.8
1933	8.2	14.6	−0.8	57.6	−57.0	52.2
1934	8.3	75.7	−3.6	30.2	18.6	−112.4
1935	8.4	29.3	−54.3	−30.7	−13.1	−123.5
1936	4.5	20.3	−19.1	44.3	22.5	−7.7
1937	7.9	−5.5	0.9	−9.5	8.3	14.3
Mean	7.6	16.8	−31.9	12.0	8.9	−0.1
Standard deviation	1.6	36.1	41.1	55.4	23.7	13.2
t	—	3.218**	5.374**	1.496	2.605*	0.008

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

TABLE II

SEASONAL RAINFALL COEFFICIENTS, SWIFT CURRENT

Year	a'	b'	c'	d'	e'	f'
1890	6.5	37.8	−12.1	36.3	1.5	−102.9
1891	9.7	9.9	−95.0	3.4	35.6	−151.6
1892	7.6	−71.0	−16.0	24.0	−1.8	−87.0
1893	3.8	33.3	−41.5	−74.7	0.1	62.1
1894	3.7	−28.1	−9.8	51.4	−12.6	7.4
1895	5.0	2.3	−73.5	45.3	24.5	32.1
1896	5.6	12.7	10.0	40.2	−33.0	−31.2
1897	6.1	69.3	−51.9	−50.7	40.3	165.9
1898	5.4	10.4	−55.4	−8.6	10.2	−1.0
1899	8.2	31.4	−101.7	−55.1	−19.5	−15.7
1900	6.5	40.4	−18.0	15.4	−16.2	34.4
1901	8.3	43.1	−48.0	89.6	45.0	33.4
1902	7.7	−33.9	−95.2	121.6	−15.4	−14.2
1903	7.9	7.5	−71.9	−15.5	−12.7	61.7
1904	5.3	23.8	−46.9	4.3	13.3	7.7
1905	7.4	−32.1	−77.2	80.4	19.0	65.4
1906	7.9	−25.8	−78.3	138.7	12.9	−204.1
1907	5.2	15.7	−26.8	−51.8	−23.0	−46.0
1908	3.5	−7.3	−39.8	18.2	9.4	−84.2
1909	9.3	−4.9	−142.4	35.6	30.6	−60.2
1910	4.8	13.0	−32.2	−28.0	1.4	−38.0
1911	6.0	8.3	−51.9	−11.7	1.1	−17.1
1912	6.0	8.4	−50.0	14.4	−17.0	12.6
1913	5.4	−2.1	−65.4	4.4	8.2	−56.0
1914	3.5	27.6	−0.4	42.6	22.6	−51.6
1915	6.2	−14.4	−62.1	106.1	−11.3	45.7
1916	7.1	18.6	−67.5	11.1	11.7	−59.7
1917	3.6	13.6	2.8	−4.8	14.0	−62.8
1918	3.4	.5	−14.1	−18.5	8.1	−30.7
1919	4.6	5.8	23.4	−72.2	−20.8	−40.0
1920	4.9	−2.0	−43.0	35.0	2.6	17.8
1921	7.0	51.6	11.9	76.1	13.3	37.9
1922	6.5	−32.9	−77.3	74.1	−7.9	−102.9
1923	7.5	−13.1	−143.6	17.4	53.6	−100.2
1924	5.8	5.7	−66.4	20.2	−17.2	−4.6
1925	6.0	14.9	31.5	−19.1	13.3	−8.5
1926	6.6	24.9	−63.1	−5.1	−24.1	−9.3
1927	10.1	10.0	−12.5	92.5	−45.9	142.7
1928	5.0	−15.3	−89.7	20.7	44.5	−71.1
1929	5.0	−10.2	−34.4	74.8	10.2	−25.0
1930	6.1	12.1	6.6	39.6	25.4	−129.6
1931	4.9	45.3	−14.1	18.3	5.3	−61.5
1932	8.1	21.4	−98.7	−126.1	17.5	56.5
1933	7.2	29.5	−19.9	−11.5	−33.1	−110.3
1934	5.0	18.5	−52.5	74.5	39.9	−111.1
1935	6.3	−18.9	−76.2	−37.4	22.0	−28.0
1936	4.0	.6	−31.5	23.1	− .7	−71.7
1937	2.4	12.7	−6.9	7.7	−3.9	19.9
Mean	6.0	7.7	−45.6	18.0	5.0	−24.7
Standard deviation	1.7	25.5	39.6	52.7	23.3	70.5
t	—	2.090*	7.973**	2.371*	1.490	2.425*

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

TABLE III

SEASONAL RAINFALL COEFFICIENTS, EDMONTON

Year	a'	b'	c'	d'	e'	f'
1890	10.0	71.2	−89.2	−13.8	5.4	37.2
1891	7.6	34.5	−98.8	−65.0	38.2	111.2
1892	5.8	17.9	−61.2	−46.6	15.2	−84.2
1893	6.9	−15.7	−82.7	53.3	19.3	73.3
1894	6.4	13.0	−49.1	78.5	−8.7	−52.7
1895	5.8	16.4	−7.0	−11.6	12.6	−37.6
1896	5.5	−17.1	−51.5	−10.1	−3.3	−24.7
1897	6.2	17.2	−91.1	−9.3	44.5	−17.7
1898	3.8	19.5	−49.5	12.5	25.1	−35.3
1899	9.0	22.7	−55.7	−99.3	−41.1	−91.5
1900	10.1	−5.6	−56.0	−53.6	−2.8	−28.0
1901	11.5	47.2	−121.0	−39.8	79.4	285.8
1902	10.9	7.0	−22.6	171.0	−37.0	163.8
1903	8.8	27.3	−83.2	−51.2	8.0	−95.2
1904	6.0	16.3	−78.3	16.3	29.7	−67.3
1905	7.3	32.3	−82.0	43.8	28.4	−18.6
1906	6.1	9.0	−59.8	56.0	29.2	−37.4
1907	7.3	26.7	−81.2	−56.8	−21.0	−57.8
1908	7.6	−6.3	−83.9	73.7	15.7	−87.5
1909	6.2	−4.0	−37.7	40.5	4.5	78.3
1910	5.2	8.9	−71.3	−36.1	−5.3	−46.1
1911	10.1	56.7	−105.8	−55.8	8.8	25.2
1912	9.3	15.6	−81.3	−76.9	−5.9	18.1
1913	8.6	42.3	−87.3	−96.7	5.5	−46.7
1914	9.3	−9.0	−154.5	57.5	39.1	−190.1
1915	9.9	47.9	−77.8	7.4	32.6	−69.8
1916	7.4	17.1	−59.7	−54.9	−6.9	−11.7
1917	6.8	51.5	−11.4	−31.0	9.2	20.8
1918	6.5	1.6	−70.1	−29.9	−1.7	−46.3
1919	5.1	−1.9	−18.6	34.6	−8.2	81.8
1920	7.4	−2.2	−66.7	49.3	9.9	−66.1
1921	6.1	20.7	−70.0	4.2	22.4	13.8
1922	5.3	9.7	−15.8	−72.8	−20.8	−17.8
1923	7.0	13.4	−91.9	−1.1	9.9	−44.5
1924	6.7	26.0	−71.1	−53.5	5.1	45.1
1925	6.0	−21.9	−15.3	−57.9	1.7	−57.3
1926	8.3	19.3	−76.8	−2.2	−41.4	−54.8
1927	7.4	28.2	−48.6	50.2	32.0	60.8
1928	7.6	24.5	−53.8	−5.0	19.2	98.0
1929	5.7	9.0	−41.9	−77.5	14.3	40.3
1930	4.7	−10.8	−53.1	12.7	1.7	−20.5
1931	9.1	26.6	−110.0	−.4	7.4	−135.8
1932	5.6	−31.6	−34.3	−14.1	23.1	50.1
1933	6.6	−.2	−74.9	14.3	18.9	16.9
1934	8.2	−8.9	−53.2	49.6	14.0	−23.2
1935	7.7	−40.1	−35.6	8.4	−8.8	−80.4
1936	5.9	−14.4	−35.6	33.6	−17.2	24.0
1937	8.2	43.3	−85.7	−70.7	36.9	244.7
Mean	7.3	13.6	−64.9	−6.8	9.1	−3.3
Standard deviation	1.7	23.1	29.9	49.8	24.4	87.2
t	—	4.067**	15.029**	0.947	2.585*	0.260

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

of each column of b' . . . f', suggest that the mean values of b', c', and e' for Winnipeg and Edmonton, and of b', c', d', and f' for Swift Current do differ significantly from zero. It is true that this test of significance can only be regarded as approximate when applied to distributions differing appreciably from normality; but the indications are that, in spite of very pronounced annual fluctuations, the average incidence of rain during the spring and summer months does follow a definite sequence at all three stations.

The second-degree coefficient, c', reflects the most pronounced feature of the average sequences, namely, a maximum incidence of rain in the middle

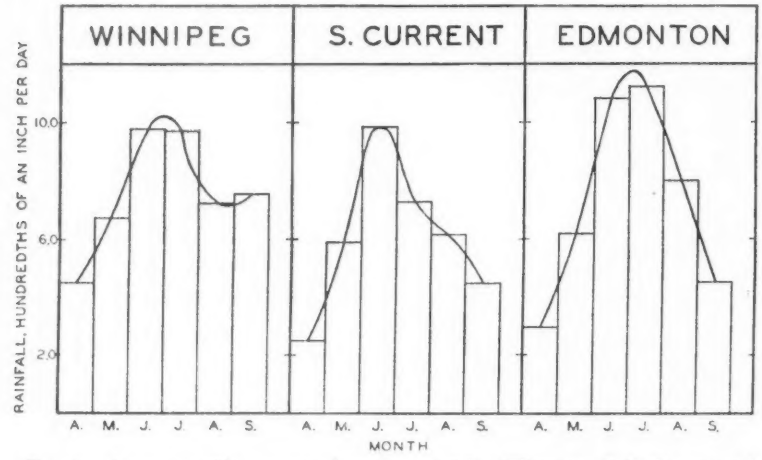

FIG. 1. *Average monthly sequence of summer rainfall at Winnipeg, Swift Current, and Edmonton, 1890–1937.*

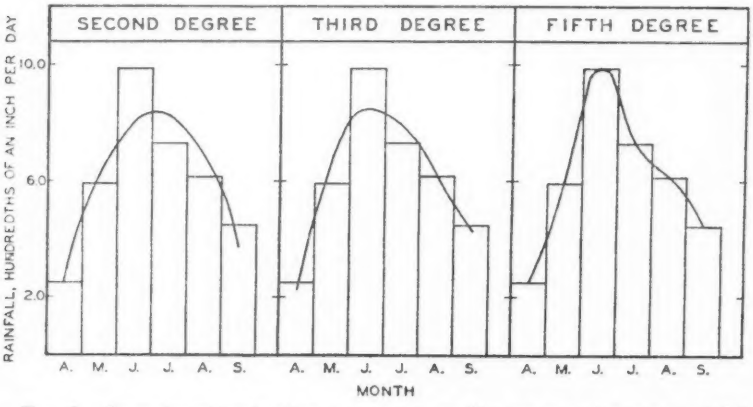

FIG. 2. *Successive approximations to average monthly sequence of summer rainfall 1890–1937 at Swift Current by polynomials of second, third, and fifth degree.*

part of the season, i.e., in June or July. This is illustrated in Fig. 1, which shows the 48-year monthly average for each location. The significance of the higher order terms d' and f' at Swift Current, and e' at Winnipeg and Edmonton, is occasioned by various inequalities in the rate of increase or decrease in precipitation in successive months. These are identifiable in Fig. 1, as well as more specifically in Fig. 2, which illustrates successive approximations to the average sequence at Swift Current by polynomials of the second, third, and fifth orders.

As might be inferred from the significance of the polynomial coefficients, the average sequence recorded at Swift Current differs from that at either Winnipeg or Edmonton, most notably in the marked decrease in rainfall in July and August. The maintenance of precipitation in September at Winnipeg is also worthy of remark.

Variance and Covariance of Annual Coefficients

As was noted in the preceding section, all of the rainfall coefficients, both of amount (a') and of distribution ($b' \ldots f'$) show large annual variations; the standard deviation of a', for example, (comprising, in a normal distribution, about one-quarter of the main range) is itself 21% of the mean at Winnipeg, 29% at Swift Current, and 24% at Edmonton. Normality of the frequency distribution of the annual values of $a' \ldots f'$ for each station was tested by calculation of the statistics g_1 and g_2 (4). In this way it was found that none of the distributions of the Winnipeg coefficients deviated from normality to an extent demonstrable in a series of 48. However, e' and f' for Edmonton, and a', b' and f' for Swift Current, all gave significantly positive values of g_2, indicative of a clustering of the majority of these coefficients in the central region of the range of variation, with, however, a small number of rather markedly aberrant individuals; whilst f' for both Edmonton and Swift Current was also characterized by a positive g_1, i.e., positive skewness.

Table IV shows the coefficients of correlation of the annual values of a' (proportional to the total seasonal rainfall) with those of the distribution coefficients $b' \ldots f'$ for each station. The correlation coefficients for Winnipeg are all statistically insignificant, from which it may be deduced that, on the average, the main features of the seasonal trend in precipitation were essentially similar in both wet and dry years at this location. This is seen to be the case from Fig. 3. In view of the deviation from normality of the frequency distribution of certain of the rainfall coefficients for Swift Current and Edmonton, "normal" tests of the significance of correlation coefficients involving these quantities cannot be exact. The indications are, however, that at Swift Current c' tended to become increasingly negative with increasing a'. This is also the case at Edmonton, but here there is in addition a moderate degree of positive association between b' and a'. At these two stations, therefore, the characteristics of the rainfall sequence must be to some extent modified in seasons of above- and below-average total precipitation. The nature of this modification is illustrated in Fig. 3. At

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN RAINFALL COEFFICIENTS OF AMOUNT (a') AND OF DISTRIBUTION (b' . . . f')

Rainfall coefficients	Coefficients of correlation		
	Winnipeg	Swift Current	Edmonton
a' and b'	−0.18	−0.05	0.43**
a' and c'	− .23	− .48**	− .49**
a' and d'	.18	.19	− .03
a' and e'	.12	.00	− .05
a' and f'	− .00	.03	.17

** *Exceeds 1% level of significance* ($r = \pm 0.37$).

Swift Current, where c′ was correlated with a', the concentration of rain in the middle of the season, with a maximum in June, is much more pronounced in the seasons of above-average totals. Both b' and c' were correlated with a' at Edmonton, and here it is seen from Fig. 3 that the excess of rain in the above-average seasons is most pronounced in July, and is also notably greater in September than in April. A qualitative, as well as a quantitative difference between wet and dry seasons at these points is thus indicated, which may be expected to affect not only the yield (7) but also the chemical composition of crops (6).

Secular Trend

In order to determine whether the variations in precipitation described above exhibited any degree of orderly sequence in time, or seemed to be

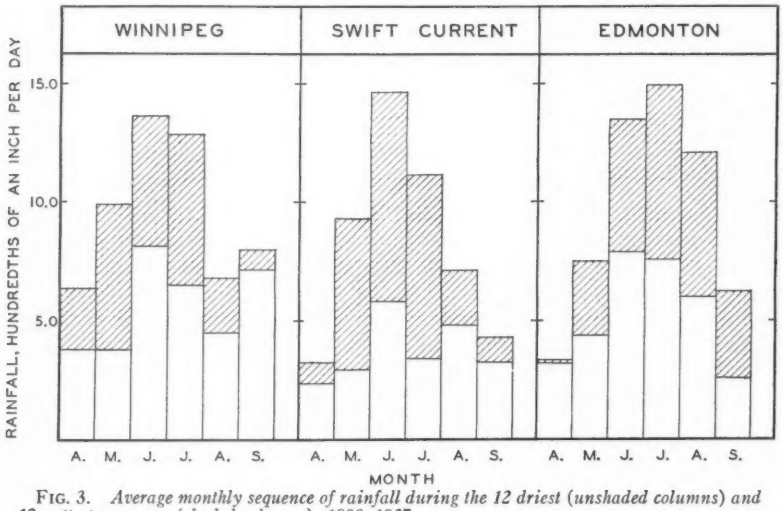

FIG. 3. *Average monthly sequence of rainfall during the 12 driest (unshaded columns) and 12 wettest summers (shaded columns), 1890–1937.*

essentially fortuitous or random in their incidence, the 48 annual values of $a' \ldots f'$ for each station were themselves subjected to a regression analysis in which a polynomial function of time of the fifth degree was fitted to each series, again by the use of Fisher and Yates's tables (5). Significance of the regression coefficients thus obtained was tested by the analysis of variance procedure (4), with the results summarized in Table V.

These indicate that none of the six coefficients for Winnipeg exhibits any demonstrable secular trend, but that both a' and f' for Swift Current do show some element of systematic variation with time. This is, however, of an

TABLE V

ANALYSIS OF VARIANCE OF RAINFALL COEFFICIENTS

Source of variance	Degrees of freedom	Mean square					
		a'	b'	c'	d'	e'	f'
Winnipeg:							
Progressive increase or decrease	1	7.11	3147	3570	3031	1289	828
Non-linear trends	4	0.88	1983	956	4290	333	3563
Residual	42	2.58	1199	1713	2957	567	9047
Swift Current:							
Progressive increase or decrease	1	4.32	6	671	121	15	3548
Non-linear trends	4	6.89*	179	1355	647	82	13514*
Residual	42	2.61	708	1612	3048	547	4194
Edmonton:							
Progressive increase or decrease	1	2.02	1987*	1128	61	202	768
Non-linear trends	4	4.24	754	1257	1281	98	6746
Residual	42	2.99	478	854	3127	557	7841

* *Exceeds mean square residual, 5% level of significance.*

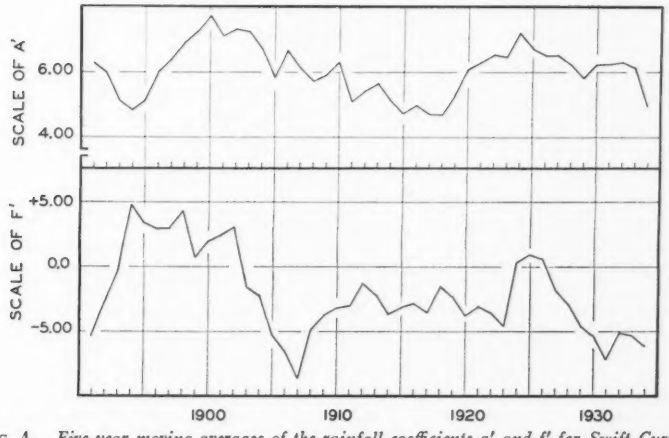

FIG. 4. *Five-year moving averages of the rainfall coefficients a' and f' for Swift Current, 1890–1937.*

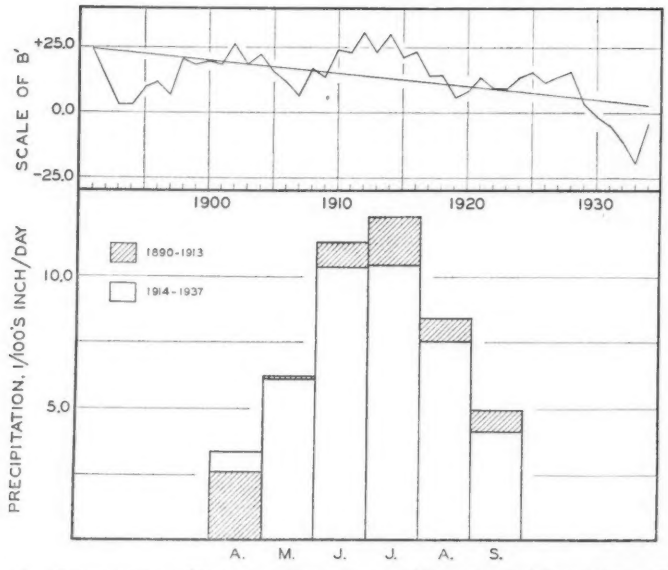

FIG. 5. *Upper portion: Five-year moving average and linear trend of the rainfall coefficient b' for Edmonton, 1890–1937. Lower portion: Average monthly sequence of rainfall at Edmonton, 1890–1913 and 1914–1937.*

oscillatory nature, neither showing any persistent tendency to increase or decrease over the 48 years as a whole. On the other hand, b' for Edmonton has on the average tended to decrease slightly during the period under review (Fig. 5), indicating some diminution in the proportion of the total precipitation occurring in the latter half of the season (July to September) relative to that falling in the months April to June. The lower portion of Fig. 5 illustrates this effect, which is the only progressive change in the seasonal distribution of precipitation demonstrable. It is noteworthy that there is no indication of any consistent increase or decrease in the total amount of precipitation recorded at any of the three stations.

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A SOLUTION FOR STAINING DIFFERENTIALLY THE SPORES AND VEGETATIVE CELLS OF MICRO-ORGANISMS[1]

By P. H. H. Gray[2]

Abstract

A solution for staining differentially the spores and vegetative cytoplasm of bacteria, yeasts, and certain fungi has been developed. The solution is a mixture of two phenyl methane dyes, malachite green and basic fuchsin; it can be used as a concentrated aqueous solution or as a dilute saline solution. It is not necessary to use heat, though heating is recommended for staining "ripe" spores of bacteria. Decolorization and counterstaining are not required. Bacterial and yeast spores are stained blue or greenish-blue, vegetative cytoplasm light violet or pink. Young bacterial cells stain a deep violet, older cells light violet; the solution can thus be used as a general stain. The acid-fast organism *Mycobacterium berolinensis* was stained greenish-blue with the granules violet. The saline solution is recommended as a differential stain with young colonies of *Aspergillus* and *Penicillium*; the terminal growing tips and young branches of hyphae are stained blue, older hyphae light violet, spores and conidiophores blue.

Introduction

The methods that have been developed for staining the endospores of bacteria, the ascospores of yeasts, and the cells of "acid-fast" bacteria, require the application of heat to the stain with subsequent decolorization by acid, acid alcohol, or sodium sulphite, followed by a counterstain. The development of a method for staining the "vegetative" cells and the spores differentially by means of a single staining solution, in one operation, may prove to be a useful contribution to laboratory methods.

The Staining Solutions

Trials were made with aqueous solutions of malachite green containing different proportions of other phenyl methane dyes of contrasting colour. A mixture of malachite green and crystal violet stained spores greenish-blue and vegetative cytoplasm violet in films of *Bacillus mesentericus*, *B. megatherium*, and *B. subtilis*. The young spores were somewhat masked by an excess of violet colour, but the excess could be removed by washing for a few seconds with 95% ethyl alcohol, or by spreading a thin film of nigrosin over the stained cells. It was found that better results could be obtained by mixing basic fuchsin with the malachite green solution; with this mixture there was a more clear-cut differentiation. The proportions of these two dyes finally adopted were such that subsequent treatment with alcohol or nigrosin could be omitted, though, as stated below, the nigrosin may greatly improve the stain of some films. Two forms of the solution have been developed: an aqueous, concentrated solution and a saline dilution prepared from that.

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Solution A. The Aqueous, Concentrated Solution

The solution is composed of the following amounts of the dyes in distilled water:

Malachite green	0.50%
Fuchsin, basic	0.05%

It has been found convenient to prepare separate solutions of the two dyes, in double strength; solution may be hastened by placing the containers in a water bath at 56° C; equal volumes of these solutions are mixed to obtain the staining solution with the dyes in the proportions stated. The staining solution appears to be stable. It should be noted that as there may be differences in dye content in dyes of different manufacture, or in different batches from a factory, a slight modification in quantities may be necessary. The malachite green used in this laboratory was supplied by The British Drug Houses, Limited, Toronto; the basic fuchsin by Coleman and Bell.

Solution B. The Saline, Diluted Solution

This is prepared by diluting two parts of solution *A* with eight parts of a 0.8% sodium chloride solution. This mode of dilution was adopted since it was found that dilution of the aqueous, concentrated solution with water, or 10% glycerine solution, rapidly altered the cellular contents of the fungi used.

Methods of Staining and Organisms Used

Aerobic Spore-forming Bacteria

The method of staining that has proved consistently to give good differentiation with *B. megatherium*, *B. mesentericus*, and *B. subtilis*, is simple: the solution is allowed to act on fixed films for 3 or 4 min. at room temperature; the excess of stain is then washed off in running water, the film dried, and examined under the oil immersion objective. Spores in the early stage of development stain blue, in older stages, greenish-blue; ripe spores within the cell are tinged greenish-blue; free spores are unstained; the cytoplasm of vegetative cells stains violet in young cells, light violet in older cells, in some species pink rather than light violet.

Another simple method is to mix the cells in a loopful of the solution, on a slide, and allow it to dry. Fixing does not appear to be necessary. The excess of dry stain is washed off. The differentiation by this method is not so clear-cut as it is by the method described above.

By staining over steam, or by heating in a flame until steam rises, a sharper differentiation is obtained. The method adopted for heating has been to place the slide, with the stain on the film, on a 100 ml. beaker containing a little water; as soon as the water boils the flame is removed and the heating continued for 1 min., though less may be all that is required; the slide is then cooled, washed, and dried. By this method (with solution *A*) the young spores may be masked by excess of violet; this may be removed by washing for a few seconds with 95% ethyl alcohol, or by covering the film with a thin layer of a sterile 2% solution of nigrosin. A loopful of the nigrosin is placed

near the film after it has been stained, washed, and dried; by means of a strip of unsized (letterhead) paper, measuring one-half inch wide by one and one-half inches long, it is drawn, with firm pressure and rapid movement over the film on which it thus dries quickly. The nigrosin (which is not recommended for films that have been stained with the saline solution) is useful to demonstrate "capsules" in young cultures in which spores have begun to develop; these have been found in *B. megatherium**. Most of the violet colour is removed by the nigrosin but the spores are left stained.

"Acid-fast" Bacteria

Films were prepared from young cultures of "acid-fast" bacteria grown on slants of glycerol agar. Both solutions stained the cells of *Mycobacterium berolinensis* (both *r* and *s* forms) greenish-blue with the granules violet.

Smears of sputum were stained and the excess of violet removed by washing in 95% ethyl alcohol; organisms that resembled *Mycobacterium tuberculosis* were seen, stained greenish-blue in the light violet background. The film was then cleansed of oil and restained by the Ziehl-Neelsen method; the same cells were identified by their position on the slide, and found to be "acid-fast" bacteria. Neither solution (*A* or *B*) gives as clear-cut a differentiation as that given by the Ziehl-Neelsen method; moreover, a number of densely stained, greenish-blue short-rod and coccoid cells were observed, that were not "acid-fast" when stained by that method.

Other Bacteria

As indicated above, solution *A* is a good general stain, differentiating young from older cells. In it, cells of *Lactobacillus acidophilus*, after growth for 24 or 48 hr. in milk, stained light violet with granules a deep violet. *L. bulgaricus* also showed variability in cytoplasmic staining.

Yeasts

Two yeasts, *Saccharomyces cerevisiae* and a *Saccharomyces* obtained from fermenting apple juice, were found to form spores rapidly if grown on slopes of Bacto tryptone glucose-extract agar at 35° C.; spores began to develop in 48 hr., and after three weeks about 80% of the cells of the latter organism had formed ascospores.

In staining, the spores and vegetative cells are adequately differentiated by mixing a loopful of solution *A* with the yeast and placing a cover glass on the suspension. Many cells are stained a deep violet; the spores are greenish-blue; but they may not all stain simultaneously. Some cells do not stain. It has been observed that by this method, only one of the two or three spores in a large number of asci were stained.

The spores are well differentiated from the other cells by mixing the cells in the staining solution *A*, and allowing the film to dry, as described above. The fixed film, heated, gives better differentiation, with either solution.

* *It has been found that broth is a better medium than water for preparing suspensions of this organism, which tends to "clump" in water.*

After staining with solution *A*, it is necessary to use nigrosin in order to delineate the cell walls.

Aspergillus and Penicillium

The saline, diluted solution *(B)*, was developed especially for use with "colonies" of these organisms grown on the surface of agar in plates. A clear medium is required; on Bacto potato dextrose agar, colonies were in suitable stages of development after incubation for 40 hr. at 35° C. *Aspergillus* is the better organism for demonstration since *Penicillium* forms a rather compact growth.

In staining, a drop of the solution is placed on a cover glass; this is inverted over the colony and pressure gently applied. The conidiophores and young spores stain greenish-blue, the terminal growing points of hyphae the same colour; older portions of hyphae become light violet, with young branches the same colour as the terminal portions. Ripe spores of *Aspergillus oryzae* remain brown with a tinge of green in the contents.

EFFECTS OF POTASSIUM ACID PHOSPHATE, CANE SUGAR, ETHYL MERCURIC BROMIDE, AND INDOLYLACETIC ACID IN A TALC CARRIER ON THE ROOTING OF STEM CUTTINGS[1]

By N. H. Grace[2]

Abstract

Greenwood cuttings of *Deutzia Lemoinei*, *Symphoricarpus albus*, and *Weigela rosea* and dormant cuttings of *Lonicera tatarica* were treated with a series of 32 talc dusts containing potassium acid phosphate at concentrations of 0, 0.1, 1.0, and 10%, in combination with 0 and 10% cane sugar, 0 and 50 p.p.m. ethyl mercuric bromide, and 0 and 1000 p.p.m. indolylacetic acid. The lower concentrations of phosphate tended to increase rooting and reduce mortality of two of the species of greenwood cuttings whereas the 10% concentration was ineffective or injurious. However, this concentration was favourable to the rooting of dormant cuttings. Indolylacetic acid treatment increased the number of rooted cuttings and the number and length of roots. Beneficial effects were indicated for organic mercury and cane sugar treatments. However, these were attributed largely to the combinations with phosphate and indolylacetic acid. The results indicate that the effectiveness of dusts containing indolylacetic acid in the treatment of plant stem cuttings may be increased by the addition of nutrient and disinfectant chemicals.

In several communications have been reported the results of experiments in which plant cuttings were treated with talc dusts containing cane sugar, an organic mercurial disinfectant, and indolylacetic acid (3, 4, 8). Both cane sugar and the organic mercurial disinfectant produced beneficial effects under certain conditions. The foregoing work has been followed up by experiments in which inorganic nutrients have been added to the dust mixture (7). This communication describes experiments in which potassium acid phosphate was added to dusts containing cane sugar, ethyl mercuric bromide, and indolylacetic acid.

Experimental

The effects of potassium acid phosphate, cane sugar, ethyl mercuric bromide,* and indolylacetic acid were investigated by an experiment of factorial design. The series of dust mixtures comprised potassium acid phosphate at four concentrations, namely, 0, 0.1, 1, and 10%, in combination with cane sugar at 0 and 10%, ethyl mercuric bromide at 0 and 50 p.p.m., and indolylacetic acid at 0 and 1000 p.p.m. (parts of chemical to a million parts of the mixture with talc by weight). The complete series of possible dosage combinations of the four chemicals required 32 different talc dusts prepared by a grinding-mix operation from master dusts (7).

The experimental design involved three completely randomized replicates of the 32 treatments with 10 cuttings per treatment group. Four plants were

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* *The ethyl mercuric bromide used in these experiments was prepared by a method developed in the Division of Chemistry, National Research Laboratories, Ottawa, by Dr. A. Cambron. This procedure yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF PLANT STEM CUTTINGS TREATED WITH TALC DUSTS CONTAINING POTASSIUM ACID PHOSPHATE, CANE SUGAR, ETHYL MERCURIC BROMIDE, AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square										
		Weigela		*Deutzia*		*Symphoricarpus*				*Lonicera*		
		Number of cuttings dead	Number of cuttings rooted and callused	Number of cuttings rooted	Weight of roots	Number of cuttings rooted	Number of roots per cutting rooted	Length of roots per cutting rooted	Mean root length	Number of cuttings rooted	Number of cuttings with new growth	Weight of new growth per cutting with new growth ($\times 10^3$)
Replicates	2	4923**	3950**	2018**	0.155	97.2	0.66	312	17.35	856.7**	294.7	9.833
Average treatment effects												
Organic mercury	1	1470*	446	1004*	0.108	582.1	0.51	233	18.64	51.3	7.9	1.584
Cane sugar	1	12	8	126	0.002	26.9	3.35	97	7.10	776.3*	1299.5**	15.251
Indolylacetic acid	1	807	2615**	5204**	1.741**	4913.5***	90.87***	9789***	0.47	8158.6**	306.7	0.001
Phosphate	3	1303**	1041**	604*	0.122	907.5**	5.94**	623	5.10	424.0*	309.0	10.504
Interactions												
Organic mercury × cane sugar	1	28	23	14	0.110	431.8	0.04	375	10.60	270.0	8.4	35.651
Organic mercury × indolylacetic acid	1	709	132	1	0.001	17.0	0.77	304	0.06	82.5	0.5	21.902
Organic mercury × phosphate	3	289	285	562*	0.085	388.2	1.09	237	8.16	199.7	197.5	33.729*
Cane sugar × indolylacetic acid	1	410	99	1386**	0.397	169.6	1.53	194	49.45*	822.5*	15.7	0.459
Cane sugar × phosphate	3	198	281	232	0.051	214.4	0.51	198	5.19	184.7	30.5	5.929
Indolylacetic acid × phosphate	3	133	44	153	0.066	259.0	1.59	541	18.49	235.9	41.1	1.612
Organic mercury × cane sugar × indolylacetic acid	1	93	485	65	0.070	34.1	0.01	68	6.35	87.8	252.2	26.334
Organic mercury × cane sugar × phosphate	3	315	523	215	0.234	78.7	0.73	114	5.43	75.1	190.0	22.584
Organic mercury × indolylacetic acid × phosphate	3	180	83	173	0.025	77.8	1.07	192	2.44	72.0	132.4	4.612
Cane sugar × indolylacetic acid × phosphate	3	106	308	74	0.032	140.3	0.63	46	4.88	261.1	62.0	33.337*
Organic mercury × cane sugar × indolylacetic acid × phosphate	3	131	184	530*	0.331*	395.9	1.08	169	1.66	80.1	113.7	4.368
Error	62	292	193	172	0.102	156.1	1.09	276	11.67	122.2	118.4	10.581

**Exceeds mean square error, 5% level of significance.*

***Exceeds mean square error, 1% level of significance.*

****Exceeds mean square error, 0.1% level of significance.*

used in the investigation, which thus required 960 cuttings of each species. Prepared cuttings* of the four species were sprayed with water (11), dusted in groups of 10, and planted immediately. Cuttings of *X Deutzia Lemoinei* Lemoine were planted August 16, 1939, and removed for examination September 15. Those of *Weigela rosea* Lindl. were planted August 16 and removed October 3. *Symphoricarpus albus* var. *laevigatus* Blake was planted September 1 and removed October 16. The greenwood cuttings of these three species were planted in brown sand in outside propagation frames in a garden. The frames were shaded with factory cotton screens. As heavy rains were encountered outside conditions were not particularly favourable. Dormant *Lonicera tatarica* L. cuttings were planted, on November 9, in brown sand in the greenhouse, under conditions that have been described (5), and removed December 20, 1939.

On removal, record was taken of the number of cuttings surviving, callused, and rooted, the number and length of roots and, for *Lonicera*, the number of dormant cuttings that produced new growth and the weight of new growth. The fresh root weight of *Deutzia* cuttings also was determined. The data were subjected to the analysis of variance procedure except in a few instances where poor rooting provided meagre data unsuited to statistical analysis. All counts of numbers of cuttings were subjected to the inverse sine transformation prior to analysis (1).

Results

Data for the analyses of variance of responses of the cuttings of the four species are given in Table I. Results for each of the four species has indicated some significant response to treatment with indolylacetic acid and potassium acid phosphate. Although effects from treatment with cane sugar and organic mercury are not so general, several are to be noted. There were also a number of interactions between the chemicals. Poor rooting of dormant *Lonicera* cuttings without indolylacetic acid treatment necessitated confining the analyses of variance of numbers and lengths of root and the mean root length to the 480 cuttings that received the phytohormone. As the only significant effect on the number of roots per rooted cutting was that attributable to cane sugar, the results of these analyses have not been tabulated. Data for some of the more important effects of treatments have been selected and are given in the following tables.

Weigela

In Table II are given data for the effects of potassium acid phosphate treatments. The data are averages for treatments with and without cane sugar, organic mercury, and indolylacetic acid. Both the 0.1 and 1% concentrations of phosphate resulted in an increase in the number of cuttings rooted and callused and also in reduced mortality, whereas the results with 10% level of phosphate did not differ from those in the controls.

* *The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.*

TABLE II

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE RESPONSES OF *Weigela* CUTTINGS

——	Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
		0	0.1	1	10	
Number of cuttings rooted and callused	Transformed	16.29	27.56	26.38	14.97	8.03
	Per cent	14.6	27.5	24.2	13.3	
Number of cuttings dead	Transformed	40.97	29.52	33.66	46.01	9.86
	Per cent	43.3	30.4	34.6	47.5	

In the absence of organic mercury about 43% of the cuttings died, whereas in its presence mortality fell to 35%. Indolylacetic acid treatment, on the average, resulted in production of 27% of rooted or callused cuttings. In its absence the value was 13%.

Deutzia

The effects of potassium acid phosphate and organic mercury treatment on rooting are indicated in Table III, in which the data are averages for treatments with and without cane sugar and indolylacetic acid. Organic mercury increased rooting when combined with the 0.1 and 10% concentrations of phosphate. The high phosphate concentration was depressing in the absence, but not in the presence, of organic mercury.

Indolylacetic acid treatment, on the average, increased rooting from 20 to 40%. Whereas both indolylacetic acid and cane sugar increased rooting when applied separately, the effect was not additive when these chemicals were combined. At the 1% concentration of potassium acid phosphate,

TABLE III

INTERACTION EFFECTS OF POTASSIUM ACID PHOSPHATE AND ETHYL MERCURIC BROMIDE ON THE ROOTING OF *Deutzia* CUTTINGS

Concentration of organic mercury, p.p.m.	Kind of data	Concentration of potassium acid phosphate in dust, %				Mean of organic mercury treatment
		0	0.1	1	10	
0	Transformed	30.1	26.7	35.4	17.5	
	Per cent	28.3	25.0	35.8	15.0	26.1
50	Transformed	34.1	42.8	29.4	29.2	
	Per cent	34.2	46.7	28.3	26.7	34.0
Means of phosphate treatment	Transformed	32.1	34.7	32.4	23.3	
	Per cent	31.3	35.8	32.1	20.8	

Necessary difference, 5% level: phosphate means, 7.57; interaction, 10.7.

organic mercury was more beneficial to rooting when in conjunction with both cane sugar and indolylacetic acid than with indolylacetic acid alone.

The average effect of indolylacetic acid treatment was to increase fresh root weight per group of 10 cuttings planted from 0.14 to 0.41 gm. Thus, whilst the number of rooted cuttings was approximately doubled, the weight of roots produced was increased about three times. The significant interaction between the four chemicals disclosed by the analysis of fresh root weights is related to the beneficial effect of organic mercury when in combination with indolylacetic acid, cane sugar, and the 1% concentration of phosphate.

TABLE IV

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE RESPONSES OF *Symphoricarpus* CUTTINGS

——	Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
		0	0.1	1	10	
Number of cuttings rooted	Transformed	60.8	55.0	56.7	46.2	7.2
	Per cent	72.5	64.2	67.9	51.7	
Number of roots per rooted cutting		3.9	3.7	3.4	2.8	0.6

Symphoricarpus

Effects of potassium acid phosphate on rooting and the number of roots are presented in Table IV, in which the data are averages for treatments with and without indolylacetic acid, cane sugar, and organic mercury. The 10% concentration of phosphate resulted in rooting percentage and number of roots per rooted cutting being below both the control groups and the groups receiving the two lower phosphate concentrations. Data for the average effects of indolylacetic acid treatment are given in Table V. The application of 1000 p.p.m. of indolylacetic acid in a talc carrier has resulted in marked increase in percentage of rooting and in both the number and lengths of root per rooted cutting.

TABLE V

AVERAGE EFFECTS OF INDOLYLACETIC ACID TREATMENT ON THE RESPONSES OF *Symphoricarpus* CUTTINGS

——	Indolylacetic acid in talc, p.p.m.	
	0	1000
Number of cuttings rooted, %	54.2	74.0
Number of roots per rooted cutting	2.5	4.4
Length of roots per cutting rooted, mm.	23.8	44.0

The mean root length averaged 9.8 mm. over the entire experiment. Indolylacetic acid and cane sugar each had a slightly beneficial effect upon this attribute when applied separately, but in combination reduced the mean root length to 8.8 mm.

Lonicera

The effects of indolylacetic acid and cane sugar treatment on the number of cuttings rooted are described in Table VI, in which the data are averages for treatments with and without phosphate and organic mercury. Whereas indolylacetic acid markedly increased rooting, cane sugar had a depressing effect in the absence, but not in the presence of indolylacetic acid.

TABLE VI

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND CANE SUGAR ON THE ROOTING OF DORMANT *Lonicera* CUTTINGS

Concentration of cane sugar in talc, %	Kind of data	Indolylacetic acid in talc, p.p.m.		Means
		0	1000	
0	Transformed	30.8	43.4	
	Per cent	28.3	47.5	37.9
10	Transformed	19.3	43.6	
	Per cent	16.3	47.5	31.9
Means	Per cent	22.3	47.5	

Necessary difference, 5% level, 6.38 (transformed data only).

Data for the average effects of potassium acid phosphate on the number of cuttings rooted are given in Table VII. An increase in rooting is suggested after treatment with 1% phosphate; after 10% phosphate the increase is significant. Stimulation of rooting of these dormant cuttings by the high level of phosphate is in marked contrast to the results with the other three species.

In the presence of cane sugar, 71% of the cuttings had new growth, as compared with 80% in its absence. A further partition of the variance shown

TABLE VII

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE ROOTING OF DORMANT *Lonicera* CUTTINGS

Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
	0	0.1	1	10	
Transformed	31.8	30.9	34.1	40.2	6.4
Per cent	31.7	30.4	35.4	42.1	

in Table I demonstrated that phosphate at the 1 and 10% concentrations reduced the number of cuttings with new growth.

Data for the weight of new growth per cutting with new growth demonstrated that organic mercury was slightly beneficial in the absence of phosphate, slightly depressing in conjunction with both the 0.1 and 1% concentrations, and highly beneficial with the 10% concentration. The interaction of cane sugar, indolylacetic acid, and potassium phosphate was such that whereas indolylacetic acid increased the weight of new growth in the absence of cane sugar and phosphate, its combination with sugar or the several phosphate concentrations reduced weight of new growth. However, the combinations of indolylacetic acid with both sugar and phosphate were all beneficial, increasing the weight of new growth.

Cane sugar treatment, averaged over treatments with indolylacetic acid and with and without organic mercury and phosphate, increased the number of roots per rooted cutting from 4.1 to 5.4.

Discussion

The results are in general agreement with those of other experiments in which the responses of stem cuttings were affected by cane sugar, indolylacetic acid, and an organic mercurial disinfectant, and in which there were interactions between these chemicals (3, 4, 6, 7, 8). It has been shown that various nutrient solution treatments of cuttings have marked effects and nutrient chemicals other than sugars have been applied to cuttings with successful results (2, 6, 9–11). These results demonstrate that potassium acid phosphate has some significant effects on rooting, both when considered alone and in combination with the other chemical factors. Although the results do not warrant any general recommendation as to the most desirable concentration of phosphate, it would appear that greenwood cuttings are somewhat more sensitive to overdosage than those of dormant plants. Organic mercury treatment appeared to be particularly beneficial in conjunction with phosphate and both phosphate and cane sugar. The interaction of these chemicals with indolylacetic acid suggests that such combinations may be of some value in the propagation of cuttings of certain plant species.

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THE ACTION OF MICRO-ORGANISMS ON FAT

III. OXIDATION AND HYDROLYSIS OF TRIOLEIN BY PURE CULTURES OF BACTERIA[1]

By C. H. Castell[2] and E. H. Garrard[3]

Abstract

The hydrolytic activity of 40 pure cultures of bacteria on triolein, as indicated by increased titratable acidity, has been recorded. Schiff and Kreis tests have been made on similar samples of triolein acted upon by pure cultures of bacteria, and the results of these tests are compared with the "oxidase reaction" of the individual cultures.

The results indicate that most of the Gram-negative lipolytic organisms also oxidize the fat, and that there appears to be a definite relation between the "oxidase reaction" of a bacterial colony and the ability of the organisms to produce oxidative rancidity.

Introduction

Of all the non-nitrogenous constituents of food, from the standpoint of microbial activity, least is known about fat. A study of the processes in the fermentation industries and a study of the carbohydrate reactions, now used extensively in bacterial classification, has greatly increased the knowledge of the decomposition of starches, sugars, alcohols, and related substances. Not only is there a fairly detailed knowledge of the chain of reactions occurring in fermentations, but a general idea of the microbial species bringing them about is also known. The same cannot be said for the action of bacteria on fat. In Bergey's manual, rarely, if ever, is oxidative or hydrolytic activity on fats mentioned, and apart from a few general and widely spread references there appears to be no place in the literature where information regarding the action of specific bacteria on fats can be obtained.

In this paper is reported the action of some 40 species of bacteria on triolein[4]. The results are given in terms of hydrolysis, as indicated by the increase in titratable acidity in the oil, and oxidation, as shown by the Schiff and Kreis tests. For the purpose of comparison, the "oxidase" test devised by Gordon and McLeod (3) was also made on each of the cultures.

Apart from the general information obtained, these tests were planned so as to form a basis for comparing various spot tests and dye reactions frequently used for detecting the lipolytic activity of bacteria.

Triolein was used in preference to one of the more complex natural fats so as to lessen the number of factors involved. It was preferred to the more

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[4] *It was originally intended that nothing but pure triolein would be used throughout these and succeeding experiments. Unfortunately, owing to the war, the source of this pure fat was stopped, and efforts to obtain it elsewhere have met with failure. Most of the available supply of the pure triglyceride has been saved for later experiments, and in some of this preliminary work, approximately pure triolein, prepared by crystallizing and filtering olive oil, has been substituted.*

available, saturated triglycerides, because of its capacity of being easily oxidized as well as hydrolysed.

Pure Cultures

Forty pure cultures of bacteria were used in the present series of experiments. The majority were obtained from the stock of pure cultures kept by Professor A. Davey, of this department. Some of the lipolytic organisms were kindly supplied by Dr. Hammer, of the Iowa State College, at Ames, Iowa. Most of the cultures were previously tested by their reactions in sugars and other differentiating media in order to verify their identity. The rather wide variety of species was used purposely in the hope of obtaining variations in lipolytic activity, as well as to determine the action of a large number of bacterial species on the fat.

Experimental Methods

Hydrolysis

In small, 50 ml. screw capped flasks, 20 ml. of triolein and 20 ml. of a 1% peptone solution were mixed and sterilized in the autoclave. These were inoculated with a loopful of actively growing organisms cultured on agar slants. The cultures were incubated at 25° C. (77° F.) and a second series, of 17 cultures was incubated at 37° C. (98° F.). Each day the flasks were shaken to thoroughly mix the aqueous suspension of bacteria with the fat. As the flasks were all tightly stoppered, and as the solution containing the bacteria was covered with a deep layer of oil, there was a tendency towards the production of anaerobic conditions. To determine whether this had any marked influence on hydrolysis of the fat, a third series of a few cultures was prepared, providing more aerobic conditions. Sections of wide glass tubing were suspended in wide-mouthed flasks so as to leave the peptone solution in the central portion exposed to the air. The cultures were incubated at 25° C. (77° F.).

After 11 and 17 days' incubation, samples of the oil were aseptically withdrawn from the flasks and titrated with N/10 sodium hydroxide in a neutral solution of equal parts of ether and alcohol, with phenolphthalein as the indicator. At least two titrations were made for each sample and, if the figures agreed, the result was recorded. A similar titration was made on a sample of the original oil. The figures in Table I represent the differences in values obtained for the inoculated samples and those of the original oil. The results are calculated in terms of oleic acid.

In some cultures duplicate tests were made; the results between similar cultures never varied more than 0.05%, the average variation being 0.04%. This was not the case when, as indicated in the table, different strains of the same culture were used.

Oxidation

The flasks for these tests were prepared in the same way as those for acid production. At the end of 17 days, a Kreis test and a Schiff test were made on each sample according to the methods given by Lea (6). As these tests

were numerous and only comparative results were required, the extent of the reactions was judged by comparing the colours and marking them from (+) to (++++) as the strength of the reaction increased. The results given are for samples incubated at 25° C. (77° F.)

The "oxidase" test was made by flooding agar plates of each culture with a weak solution of *p*-aminodimethylaniline monohydrochloride, as described in a previous paper (1).

Results and Discussion

The most significant results from these experiments are given in Table I. Of the 40 cultures tested for acid production at 25° C. (77° F.) only three produced more than 1% acidity; 11 produced more than 0.1%; 26 produced no acidity, and the remaining three less than 0.01%. Besides those listed in Table I, the following organisms produced no acid: *Bacillus subtilis*, *B. panis*, *B. graveolens*, *Salmonella enteritidis*, *S. aertrycke*, *Escherichia coli communior*. *Rhizobium trifolii*, and *Brucella abortus*. Several of these included two strains.

In the samples in which the oil was prevented from completely covering the surface of the peptone solution, there was a marked increase in the amount of fatty acid formed by some species. The most notable example was *Staphylococcus aureus*, which, under these conditions, produced 11.97% acid (10.33% more than in the regular samples); *Bacillus mesentericus* produced 0.29% acid (0.24% more) but *Alcaligenes viscosus*, on the other hand, produced slightly less acid under these conditions. It is probable that these results are owing mainly to differences in growing conditions for the bacteria.

It is interesting to note that an increase in temperature had a different effect on the amount of acid produced by different cultures. *Pseudomonas aeruginosa* (*A*29) and *S. aureus* show this difference well. Both have an optimum temperature of 37° C. (98° F.), yet when the acidities at 25° C. (77° F.) and 37° C. (98° F.) are compared, it is seen that there is a marked decrease in the acid production by *P. aeruginosa* at the higher temperature, whereas there is an equally marked increase by *S. aureus*. As shown by the Schiff and Kreis tests and the "oxidase" reaction, *P. aeruginosa* has a marked oxidizing action whereas *S. aureus* is among the least active in this respect. It is probable then that the differences noted above are owing to the increased oxidation by the enzymes of *P. aeruginosa* at the higher temperature.

In general it may be stated that, of the organisms tested, species of the *Pseudomonas*, *Phytomonas*, *Alcaligenes*, and *Achromobacter* genera and *S. aureus*, were the only organisms showing any distinct lipolytic action on triolein. There is as much variation between different strains of the same species as between different species, and temperature besides other cultural conditions has a marked effect on the amount of acid formed.

It should be realized that the lipolysis indicated by these tests refers only to triolein. Some of these organisms may produce butyrases capable of hydrolysing triglycerides of lower molecular weight and other simple fatty substances.

TABLE I

RESULTS OF THE KREIS AND SCHIFF TESTS, THE INCREASED TITRATABLE ACIDITY IN SAMPLES OF TRIOLEIN MIXED WITH A PEPTONE SOLUTION, INOCULATED WITH PURE CULTURES OF BACTERIA, AND INCUBATED FOR 17 DAYS AT 25° C., AND THE OXIDASE REACTION OF THESE SAME ORGANISMS AFTER 36 HR. ON STANDARD BEEF AGAR

Organism	Strain No.	Oxidase reaction	Kreis	Schiff	Acidity (% oleic acid)	
Pseudomonas						
aeruginosa	*A*29	+++	+++	++++	1.07	(0.18)*
aeruginosa	*A*29*a*	+++	++++	++++	0.73	
fluorescens	3	+++	+++	++++	0.51	
fluorescens	*M*25	+++	++++	++++	1.30	
mucidolens	6	+++	+++	+++	0.56	
fragi	5	++	++	++	0.18	
Pytomonas						
campestris	62	++	+++	+++	0.35	
Alcaligenes						
faecalis	*A*4*a*	++	Omitted	++++	0	
lipolyticum	8	+	Omitted	++	0.12	
lipolyticum	1	++	Omitted	++++	0.11	
Achromobacter						
putrefaciens	2	+++	Omitted	++++	0	
Erwinia						
caratovora	*P*3*a*	+	Omitted	+++	0	
Rhizobium						
leguminosarum		++	Omitted	+++	0	(0)*
Bacillus						
mesentericus	18	+(?)	++	+++	0.05	
cereus	19	—	+	+	0	
mycoides	*M*9	—	+	+	0	
Proteus						
vulgaris	*A*28	—	+	+++	0.05	
Alcaligenes						
viscosus	*M*4	—	+++	++	0.70	
cloaceae	*A*2	—	+	+++	0	
aerogenes	*M*3	—	+	++	0.09	(0.05)*
Escherichia						
coli	*M*15	—	+	++	0	(0)*
Salmonella						
pullorum	17	—	+	++	0	(0)*
suipestifer	*A*41	—	+	+++	0	(0.05)*
Staphylococcus						
citreus	*A*48	—?	++	+++	0	
aureus	*A*47	—	+	+	1.64	(7.83)*
Diplococcus						
capsulatus	32	—	+	+++	0	

NOTE: *The number of (+) signs indicates the strength of the reaction, and (−) indicates no reaction. In the Kreis and Schiff tests the uninoculated controls were designated (+) and the others compared to these.*

* *The figures in parentheses represent the acidity produced by the same cultures when incubated at 37° C.*

Oxidation reactions are much more complex and very much more difficult to measure than simple hydrolysis of the fat. The variations in the types of reaction and the multiplicity of products formed, produce results that cannot be estimated accurately by two simple tests. For that reason, it is realized that the results shown in Table I have a limited significance. In a

general way, however, it appears that organisms of the *Pseudomonas*, *Achromobacter*, *Alcaligenes*, and *Phytomonas* genera very actively catalyse oxidation of triolein and that most of the cocci and the bacilli tested are less active in this respect. Of these two groups, *Staphylococcus citreus* and *B. mesentericus* are apparently slight exceptions.

It is particularly interesting to note the close similarity between the results of the Kreis and Schiff tests and the "oxidase" reactions. There are a few exceptions to this. *Proteus vulgaris*, *Aerogenes cloaceae*, and one or two others that are oxidase-negative, gave a rather strong Schiff reaction, although the results of the Kreis test was almost the same in the controls.

These results agree substantially with those of other workers who have used various substrates and different methods for determining the lipolytic activity of bacteria. These have been reviewed by Jensen and Grettie (5) and Lea (6). The following authors also present confirmatory evidence; Collins (2) and Hussong (4) using a butterfat emulsion in agar as a substrate and Nile blue sulphate as an indicator, found *Pseudomonas fragi*, *P. fluorescens*, *P. mucidolens*, *Achromobacter lipolyticum*, and *A. conii* to be strongly lipolytic. Wells and Corper (8) by extracting lipase from bacteria and using ethyl butyrate, triacetin, and olive oil as enzyme substrates found *Staphylococcus* species and *P. aeruginosa* to be the most lipolytic of a number of organisms tested. Trussel and Weed (7) found 38 different strains of *Staphylococcus* to be lipolytic and that under aerobic conditions these organisms produced much more acid than when the air was excluded.

One chief difference noted was that *Escherichia coli* was frequently described as being lipolytic. In this connection it may be stated that the authors, using oil emulsion methods over a period of years have found considerable variation in the hydrolytic activity of what were considered to be cultures of *E. coli*, *Aerogenes aerogenes*, and *A. cloaceae* from different sources.

Finally, the results recorded here confirm the observations made by Jensen and Grettie (5), namely, that many of the most actively lipolytic bacteria were also able to bring about oxidation reactions in fat.

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THE ACTION OF MICRO-ORGANISMS ON FAT

IV. OBSERVATIONS ON THE CHANGES PRODUCED IN GLOBULES OF TRIOLEIN BY PURE CULTURES OF BACTERIA[1]

By C. H. Castell[2] and E. H. Garrard[3]

Abstract

A series of observations have been made on the action of 60 cultures of bacteria on globules of triolein in oil emulsion agar media. Lipolytic activity as indicated by the colour reactions of Nile blue sulphate and methylene blue and the blue soap formation with copper sulphate has been shown to coincide with that of the same organisms as measured by the titratable acidity they produced in larger samples of the oil. Other Eh indicators have been shown to colour the globules around lipolytic colonies.

Other changes in the colour and texture of the globules have been shown to coincide with the oxidative activity of the bacteria as measured by Kreis and Schiff tests on larger samples as well as by the oxidase reaction of the bacterial colony. Preliminary hydrolysis appears to hasten and intensify the oxidative reactions as indicated by the globules.

Other colour reactions in the globules and the formation of various types of crystals have been observed and their significance discussed.

Introduction

The most frequently used method of detecting the lipolytic action of bacteria is to observe the changes produced by the organisms in minute globules of fat or oil suspended in an agar base. To this oil emulsion agar various indicators may be added to aid in observing the changes that occur in the fat globules. The indicators fall into four groups: those indicating changes in pH; those measuring changes in Eh; those considered to be specific for fatty acids; and those that unite with the fatty acids, forming coloured substances such as the blue soaps formed when solutions of certain copper salts are added. Even without the addition of indicators, sufficient changes occur in the texture of some types of fat globules to indicate what is considered to be lipolytic action. The mechanism and significance of some of these reactions have been discussed by the authors in previous papers (1, 2, 3).

In the preceding paper of this series (4), the hydrolytic and oxidative action of 40 species of bacteria on triolein was recorded. In this paper, observations have been made on the action of these same cultures on globules of triolein in an agar base, using several different indicators. Besides the 40 cultures of the previous experiments, additional strains of many species have been used.

Materials and Methods

The triolein used in these experiments was manufactured in England and procured through the British Drug Houses, Limited. The agar and the

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nutrients for preparation of the media were all Difco products. Unless otherwise mentioned, the dyes and indicators were standard B.D.H. products.

The media used throughout the experiments were prepared in a manner similar to that ordinarily used in the fat emulsion agar technique. One per cent of oil was added to an agar base and sterilized in the autoclave for 15 min. at 15 lb. pressure. In some media the indicator was added before sterilization; with others it was flooded over the surface of the agar after the plates had been incubated. After the sterilized medium had cooled sufficiently, it was shaken vigorously to disperse the fat in the form of fine globules and then poured into sterile plates. Inoculation was made by streaking the surface of the solidified agar with organisms from actively growing cultures. All examinations were made through the low power lens of the microscope, although in some cases changes were evident to the naked eye.

In the first series, each of the 60 cultures were used to inoculate each of the following media:

(1) Triolein + plain agar (no added nutrients) + methylene blue
(2) Triolein + plain agar (no added nutrients) + Nile blue sulphate
(3) Triolein + standard beef extract agar + methylene blue
(4) Triolein + standard beef extract agar + Nile blue sulphate
(5) Triolein + standard beef extract agar

The dyes were added as aqueous solutions in amounts to make their concentration in the medium 1 : 40,000. The plates to which dyes were not added were prepared in quadruplicate. These were later flooded with *(a)* a saturated solution of copper sulphate, *(b)* a 0.4% solution of *p*-aminodimethylaniline monohydrochloride, and *(c)* Schiff's reagent. The fourth set was left completely untreated.

All plates were incubated for 8 days at 25° C. (77° F.). Observations were made daily on all plates except those that were to be flooded on the eighth day.

This whole series of 480 separate tests was repeated three times.

General Observations on Changes Occurring in the Inoculated Globules of Triolein

Careful microscopical observations demonstrated a remarkable variation in the form and colour of the disintegrating fat globules. Continued observation showed that many of these variations were fundamentally the same, being different stages in a few general types of breakdown. Among the factors influencing the rate of change in the globules, are their size (the smaller they are, the faster they change), their position in relation to the bacterial colony, and their relation to the surface of the medium. Many organisms produce changes, in the globules surrounding the colony, that differ markedly from the changes in globules in actual contact with the bacteria; some cause changes only in the globules that are in actual contact with the colony.

Brief descriptions only of the main types of changes that occur in the globules will be given; the results of the action of individual cultures on the

globules will be tabulated in terms of these changes. They are subdivided into changes in colour and changes in form and texture of the globules. These relate specifically to triolein, but, in general, occur in a similar way in most other fats.

Changes in Colour and Form

Normal Blue

This refers to globules in media containing either Nile blue sulphate or methylene blue. The first dye stains normal globules of triolein from pink to rose; the second does not stain them. As lipolysis proceeds, those stained with Nile blue sulphate gradually change through shades of mauve and purple to deep blue. With methylene blue they simply become uniformly blue. As can be seen in Table I, these colour reactions parallel the formation of

TABLE I

REACTIONS OF GLOBULES OF TRIOLEIN INOCULATED WITH PURE CULTURES OF BACTERIA, WITH SEVERAL DIFFERENT INDICATORS

Name of organism	Number of strains used	Colour changes							Changes in form		
		Blue, N.B.S.	Blue, M.B.	Blue droplets, M.B.	Red, dimethyl dye	Blue, dimethyl dye	Brown, dimethyl dye	Blue, C.S.	Projections	Crystals	Decolorization and granulation
Pseudomonas											
aeruginosa	5	+++	++	−	+	+	+	+	+	+	+++
fluorescens	2	+++	++	−	+	+	+	+	+	+	+++
mucidolens	1	+++	++	−	+	+	+	+	+	+	++
fragi	1	+++	++	−	+	+	+	+	+	+	++
Alcaligenes											
lipolyticus	1	+++	++	−	+	+	+	+	+	+	+
viscosus	2	+++	++	−	−	−	+	+	+	+	−
faecalis	2	−	−	−	+	−	−	−	−	−	−
Achromobacter											
lipolyticum	1	++	++	−	−	+	+	+	+	+	−
Phytomonas											
campestris	1	++	+	−	+	+	−	+	+	+	+
Bacillus											
subtilis	1	−	−	+	−	−	−	−	+	−	−
mycoides	1	+?	+?	+	−	+	−	+	+	−	−
panis	1	−	−	+	−	−	−	−	+	−	−
cereus	1	+?	+?	+	−	+	−	+	+	−	−
graveolens	1	−	−	+	+?	−	−	−	+	−	−
mesentericus	1	−	−	+	+	−	−	−	+	−	+?
Staphylococcus											
citreus	1	−	−	−	−	−	+	−	+	−	−
aureus	1	++	++	+	−	−	+	+	+	+	−

NOTE: (+++) = *strong reaction;* (++) = *moderate reaction;* (+) = *weak reaction;* (−) = *no reaction.*

N.B.S. = *Nile blue sulphate;* M.B. = *methylene blue;* C.S. = *copper sulphate; dimethyl dye = p-aminodimethylaniline monohydrochloride.*

blue soap in similar plates flooded with saturated copper sulphate solution. By comparing organisms bringing about this "normal blue" reaction with those organisms shown in the preceding paper of this series (4) to be capable of hydrolysing triolein, its significance is at once apparent. This normal blue colour in globules stained with methylene blue, Nile blue sulphate, or flooded with copper sulphate solution is indicative of hydrolysis of the fat. In previous papers (1, 3) the authors have shown that besides methylene blue, many other dyes can replace the Nile blue sulphate, the results being the same. Contrary to general belief, these colour reactions are not similar to those obtained with ordinary pH indicators, but are brought about by the extreme solubility of the oxidized form of many Eh indicators in fatty acids and the tendency of fatty acids to oxidize these dyes.

The enzymes bringing about hydrolysis, as indicated by these colour reactions, are able to diffuse through the agar to affect globules remote from the colonies. With strong lipase formers, such as some of the members of the genus *Pseudomonas*, or *Staphylococcus aureus*, this area of diffusion may extend between 1 and 2 cm. beyond the colony.

False Blue

This has been observed only in media containing Nile blue sulphate and then, only when the medium was lacking all nutrients other than fat. The globules surrounding the colony change from pink to delicate shades of blue or bluish-green. It usually occurs only after 5 or 6 days' incubation. Apart from the unusual conditions under which it occurs, it is not likely to be confused with the normal reaction of lipase formers with this dye. No matter how long the incubation continues the colour remains a delicate shade, in marked contrast with the intense dark purples and blues in globules undergoing hydrolysis.

This false blue was observed in cultures of *Escherichia coli* and *E. coli communior*, *Aerogenes cloaceae*, and the following members of the *Salmonella* genus, namely, *S. schottmülleri*, *aertrycke*, *columbensis*, *hirschfeldii*, *morganii*, *suipestifer*, *paratyphi*, and *enteritidis*. It did not occur with three strains of *S. pullorum*.

This colour reaction appears to be owing to the reducing activity of the bacteria concerned. Castell and Bryant (2) have shown that a blue colour in globules stained by Nile blue sulphate may be derived from either of two sources; it may be caused by the absorption of the aqueous blue portion of the dye when fatty acids or some other similar substance are present in the fat or it may be produced by a *reduction* of the pink fat-soluble portion of the dye. The tests for lipolysis in the preceding paper indicated that these organisms did not hydrolyse triolein and therefore the blue colour could not be caused by the presence of fatty acid in the globules. On the other hand, tested with *p*-aminodimethylaniline monohydrochloride and similar indicators, not only are the colonies of these organisms oxidase-negative, but they have a reducing action on these dyes in areas near those containing the false blue

globules. These organisms are also known to produce a relatively low potential in media in which they are growing. It is most probable then, that the blue in these globules is the result of the strong reducing activity of these particular bacteria.

Blue Droplets (Fig. 1, Plate I.)

These appear to occur only in media containing methylene blue. Instead of becoming uniformly blue, the globules contain minute blue droplets. These frequently increase in size until they occupy a large portion of the globules. The blue liquid appears to be insoluble in the fat, but readily diffuses into the agar when it comes in contact with it. These occur only in globules that are in contact with the bacteria. They were present in plates containing the following aerobic spore formers: *B. subtilis*, *B. graveolens*, *B. cereus*, *B. panis*, *B. mycoides*, and *B. mesentericus* and they were present, though not very abundant, in plates of some of the cocci. In some instances, organisms that were lipolytic showed indications of the formation of these droplets but they always became obscured as hydrolysis progressed in the globule. Table I shows only those cultures that produced the droplets not obscured by subsequent lipolysis.

The presence of these "blue droplets" in the globules in media to which methylene blue was added, raises several questions apart from their identity. Methylene blue is not generally considered to be soluble in fat. How then, does the colour get into the isolated little drops in the interior of the globule? It was observed that if the droplets came in contact with the agar they readily diffused out of the fat so that this could not be the source of the stain. The possible explanation may be contained in an observation of Michaelis (6) "Even with methylene blue, it is somewhat disturbing that the free base is very difficultly soluble". It may be that small particles of this reduced dye are suspended in the fat before the agar has solidified. Castell and Bryant (2) have previously noted that certain fat-insoluble acids (such as lactic acid) when mixed with the fat and shaken with reduced methylene blue, were able to oxidize the dye. At that time it was predicted: "peroxides and some of the carboxy acids might be the cause of isolated blue droplets in globules. As they are not fat-soluble, the blue they abstract will not diffuse throughout the whole globule but remains in isolated areas". Incidentally, it has been observed by the authors that these blue droplets are much more abundant and occur in cultures of a great many more species of bacteria when the triolein has been replaced by butterfat or certain other natural fats and oils.

Pseudo-oxidation Reactions

These occur in plates flooded with Schiff's reagent or *p*-aminodimethylaniline monohydrochloride. These dyes in their reduced or colourless form are insoluble in fat but are readily soluble when they are oxidized to a red colour. If a solution of the reduced dye is poured over an inoculated plate, certain colonies readily oxidize the dye, rendering it fat-soluble, with the result that globules surrounding these colonies soon become coloured. This occurs

in cultures of all those organisms previously shown to be "oxidase-positive", and to some extent in cultures of some other organisms. Although it has been shown that there is a close correlation between those organisms that oxidize triolein and those that are oxidase-positive, it appears that in this colour reaction the bacteria act on the dye rather than on the fat. As the dye solution in other portions of the plate becomes slowly oxidized by the atmosphere, all the globules become coloured in a similar way. It would appear that the addition of fat globules to the media used for detecting "oxidase"-producing organisms would enhance the delicacy of the test.

Apart from their pseudo-oxidation reactions, both of these indicators have shown other colour changes in globules acted upon by lipolytic colonies. The Schiff reagent produces a darker purplish-red colour, the dimethyl indicator—yellow, brown, black, blue, or green. The significance of these colour variations has been treated in a separate paper (1). It is sufficient to observe here that they all appear to be associated with fatty acid production.

Decolorized Globules and Granulation

This refers mainly to globules in media containing either Nile blue sulphate or methylene blue and occurs only in globules that are in immediate contact with the bacteria. With some lipolytic organisms, after the deep blue stage has been reached, further activity completely removes the colour. This always appears to be accompanied by changes in the texture of the globules. From a liquid or semi-liquid, they change into an amorphous granular material. The size of the particles and their form varies considerably, but they are characterized by the absence of anything similar to the typical long thin fatty acid crystals or the feathery crystals of fats (Fig. 7).

The most pronounced reaction is produced by the strongly oxidase-positive organisms, especially strains of *Pseudomonas aeruginosa* and *P. fluorescens*. In contrast, globules acted on by less strongly oxidase-positive bacteria, such as *Alcaligenes lipolyticus* or *Achromobacter lipolyticum* retain the blue colour for a much longer period and those in cultures of oxidase-negative *Alcaligenes viscosus* and *S. aureus* retain the blue colour indefinitely.

When the non-lipolytic, oxidase-positive and oxidase-negative organisms were first examined they apparently showed little action on the globules as compared to those described above. However, many of the plates were examined more than two weeks after inoculation and in cultures of *all* oxidase-positive organisms and some that were oxidase-negative a somewhat similar granular material was present. The oxidase-negative group consisted mainly of the Gram-negative rods. The reaction did not occur in cultures of cocci nor bacilli except *B. mesentericus*. It is significant that the extent of these reactions corresponds closely to the oxidation reactions determined by chemical tests in the preceding paper. One noticeable difference, however, is that where lipolysis occurs, this apparent oxidation reaction occurs in the globules very much earlier and more vigorously.

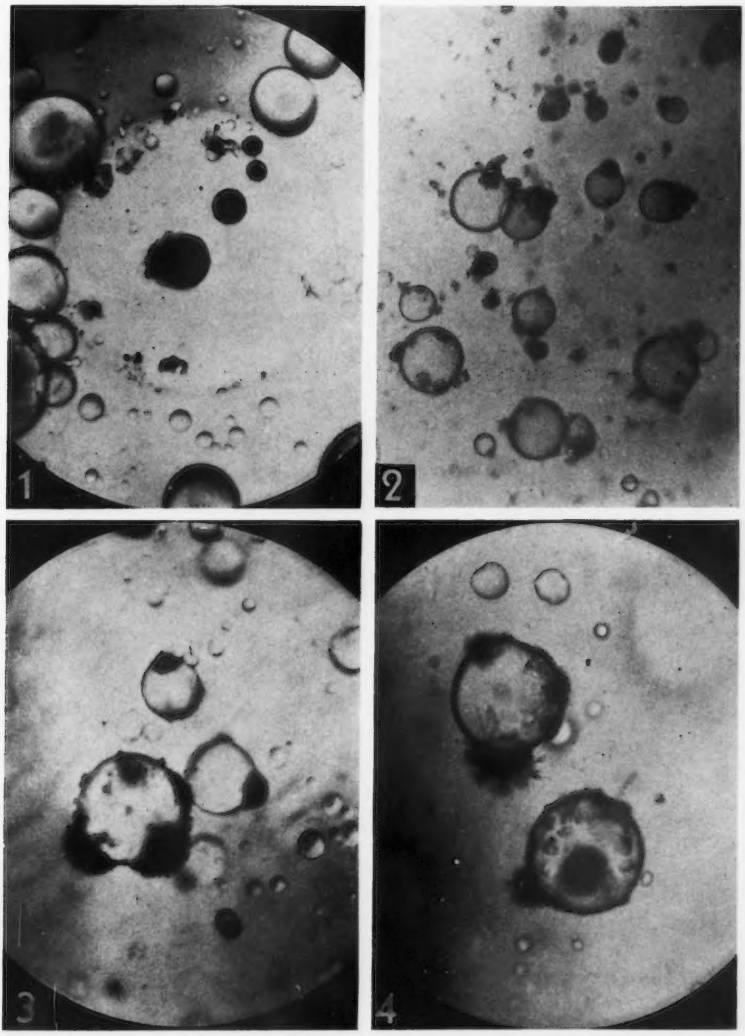
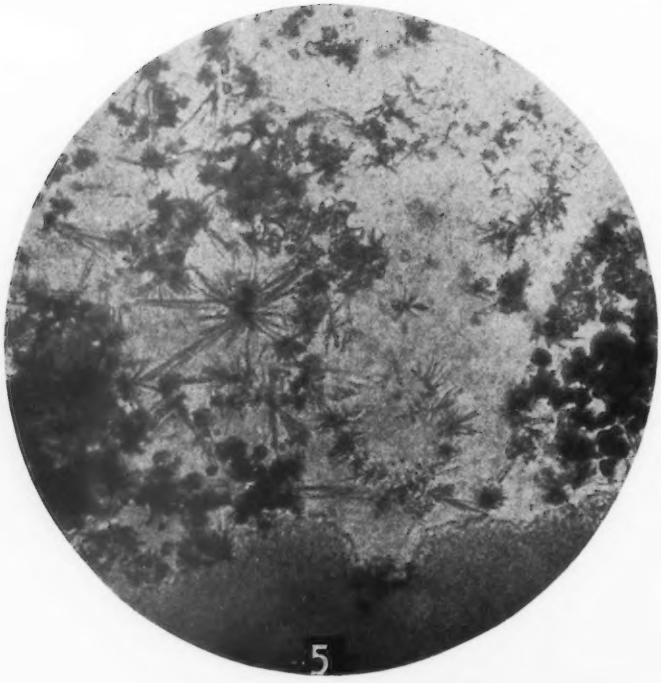
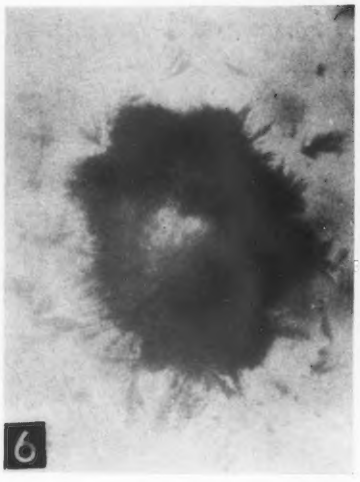


FIG. 1. *"Blue droplets" in a large surface globule of triolein in a medium containing methylene blue and inoculated with B. graveolens.* × *180.*

FIGS. 2–4. *"Projections" on globules of triolein. Fig. 1 shows general appearance of globules close to, but not touching the colony.* × *69. Figs. 3 and 4 show the crystals in greater detail. The main portion of these globules did not give any of the ordinary reactions for fatty acids.* × *180.*





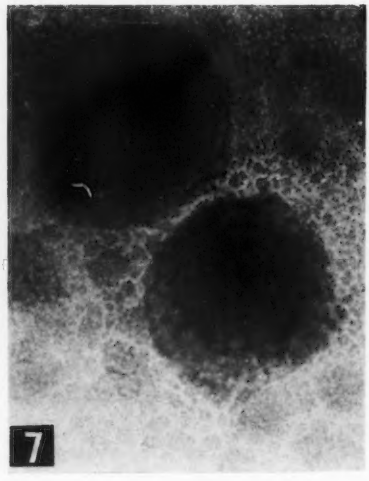


FIG. 5. *Edge of colony of P. fluorescens and adjacent, decomposing fat globules showing typical fatty-acid-like crystals. Note the marked tendency of the crystals to grow out into the medium.* × *80.*

FIG. 6. *Decomposing fat globule deep in the medium under a colony of A. viscosus. Many of the acid-like crystals have formed in the agar away from the globule.* × *160.*

FIG. 7. *Granulation of globules of triolein which have been covered with a colony of P. fluorescens.* × *80.*

Several series of tests were made substituting oleic acid for the triolein. It was found that the oxidase-positive bacteria brought about rapid changes in the acid globules and that similar, though frequently much less change, was caused by some of the oxidase-negative, Gram-positive organisms. The least change was caused by members of the bacillus and the coccus groups.

Projections (Figs. 2-4, Plate I)

These changes are more easily observed than they are described. The chief characteristic is the formation of peculiar protrusions or swellings that form on the exterior of the globules. They are composed of radiating crystals, sometimes loosely, and sometimes tightly packed. They are coloured blue by Nile blue sulphate, methylene blue, or copper sulphate solutions. Occasionally they give a sharp colour reaction with these indicators while the globule to which they are attached remains unchanged. They are formed on globules both in contact with and away from the colonies.

As well as occurring in all cultures of lipolytic organisms, they appeared also on globules in the plates containing each of the bacilli tested, and occasionally in plates of some of the non-lipolytic cocci (*Sarcina aurantiaca* and *Staphyloccus citreus*). Incidentally, it was noted that some of the common varieties of actinomycetes that were contaminants on discarded plates, produced these formations in a very marked manner without apparently having any other effect on the fat.

No explanation can be offered concerning the nature of these crystals other than that they are acid in reaction and appear to be extremely similar to crystals of myristic acid prepared by mixing the acid in a matrix of triolein and dispersing it as globules in an agar medium. (*See photomicrographs in* (2).)

Acid Crystals (Fig. 5, Plate II)

Many globules undergoing hydrolysis, as indicated by the dye reactions, produced a wide variety of needle-like crystals. These usually protruded out into the agar from the disintegrating globule, but occasionally were observed scattered in the agar surrounding the globules as though they had formed from some substance that had diffused away from the fat (Fig. 6, Plate II). In some instances it was difficult to distinguish these from the preceding type of crystal. However, the so-called acid crystals invariably were accompanied and preceded by hydrolysis of the fat as indicated by the dye reactions. The best samples of these crystals were found in cultures of *S. aureus* and *A. viscosus*. They were present, however, in all cultures of lipolytic organisms and in none of the non-lipolytic bacteria. The significance of these crystals, like those of the preceding type, can only be inferred. Their general appearance, their tendency to protrude out into the agar from the globules, and their reaction to copper sulphate, Nile blue sulphate, and other dyes and indicators all strongly suggest that they are fatty acids. How solid fatty acids could be formed by hydrolysis of pure triolein or the products of this reaction is a matter of conjecture. It may be mentioned, however, that by flooding the

plates with suitable oxidation-reduction indicators, these crystals are formed only in a reducing environment.

Effect of Added Nutrients on Lipolysis

With one or two probable exceptions there was evidence of growth on all the plates to which nutrients had been added. On plain agar, even after 20 days' incubation, the growth was meagre, or in many cultures, absent. Most of the organisms having a marked action on the fat in plates containing nutrients, showed a similar but very limited action on the fat in the plates containing only plain agar. As was mentioned before, the "false blue" reaction occurred only in those plates containing plain agar.

Reaction of other Eh Indicators in Globules of Triolein Inoculated with Bacteria

In previous papers, Castell and Bryant (2) and Castell and Garrard (3), have shown that many common Eh indicators react like methylene blue inasmuch as, although they are not fat soluble, they are readily soluble in fat containing small percentages of fatty acids. Triolein agar plates previously inoculated and incubated for 48 hr. were flooded with seven of these dyes. The dyes were used as 1% aqueous solutions. Shortly after flooding, the plates were washed off with water and the globules examined microscopically.

Of the four organisms used for inoculation, two were lipolytic and two were not and one out of each of these pairs had a strong oxidizing action on the fat. From the results of these observations (Table II) it is apparent that these particular oxidation-reduction indicators react like methylene blue. Coloration of globules surrounding a colony indicates hydrolytic action and has nothing to do with the oxidizing activities of the bacteria.

TABLE II

Absorption of Eh indicators by globules of triolein that were previously acted upon by bacterial cultures

Dye solution	Colour in the globules			
	A. lipolyticum	*P. fluorescens*	*B. mesentericus*	*Diplococcus capsulatus*
Malachite green	Blue	Blue	Colourless	Colourless
Methyl violet	Red	Red	Colourless	Colourless
Indigo carmine	Colourless	Colourless	Colourless	Colourless
Basic fuchsin	Red	Red	Colourless	Colourless
Neutral red	Red	Red	Colourless	Colourless
Rose bengal	Red	Red	Colourless	Colourless
Gentian violet	Purple	Purple	Colourless	Colourless
Methylene blue	Blue	Blue	Colourless	Colourless

Discussion and Summary

The observations described in this paper indicate that bacteria bring about several distinct changes in globules of triolein. By comparing these changes and the organisms that produce them with the results of chemical tests reported in the preceding paper it is apparent that one group of changes is associated with lipolytic organisms and the other with bacteria that oxidize either the fat itself or the product of its hydrolysis. Some organisms, especially members of the *Pseudomonas* genus are able to bring about both these reactions.

The action of lipolytic bacteria in globules of triolein is indicated, first by their intense colour reaction when stained by Nile blue sulphate, methylene blue, and other Eh indicators or saturated copper sulphate solution, and second, by the formation of fatty-acid–like crystals. The oxidizing bacteria decolorize stained fat that has been previously hydrolysed and at the same time change the globule from a liquid or semi-liquid to granular material. A somewhat similar, but retarded and much less extensive change occurs in globules not previously hydrolysed.

These observations appear to be in general agreement with those on the bacterial action on fat as described by Frazier (5): "The chief action of bacteria on fats is a hydrolytic cleavage by means of lipase, in which the fats are split into glycerine and fatty acids. Glycerine is readily acted on by a number of bacteria and is usually the first of the split products to be attacked. The course of the fermentation of glycerine will depend upon the organisms present and the general conditions but it is generally changed into lactic acid, volatile acids, and aldehydes. The fatty acids which result from the hydrolysis of the fats or the fermentation of the glycerine are usually not attacked until the more available glycerine is destroyed. Then, however, these acids may be further split by some types of bacteria into simpler acids or to CO_2 and water. The first part, then, of the action of bacteria on fat is usually hydrolysis and this is followed by oxidation processes".

Several false colour reactions, apparently indicating lipase action, have been observed and the conditions described under which they occur.

Observations have also been made on other types of change occurring in globules caused by bacterial activity; their significance is still unexplained.

It is interesting to note that there are two general "spheres of influence" in which the same bacterial colony may affect globules in an entirely different manner. These are the areas out around or deep under the colony, and the section immediately in contact with the bacteria. Many changes, including those considered to be oxidation reactions, occur only when the bacteria are in contact with the globules, whereas all those considered to be associated with lipolysis can occur in globules a considerable distance away from the nearest organism. This would suggest that one type is caused by a diffusible enzyme (lipase) and that the other is a reaction occurring at the cell surface. It is, further, interesting to note that when certain oxidation-reduction indicators are flooded over these colonies, the area in the medium where lipolysis

occurs reduces the dye but it is oxidized by contact with the colony itself. This is shown by a coloured colony surrounded by a clear zone in the medium. Similar dyes poured on colonies (*S. aureus*, *A. viscosus*, etc.) result in a colourless colony surrounded by an area of reduction.

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ENVIRONMENTAL REACTION OF PHYSIOLOGIC RACES OF *PUCCINIA TRITICINA* AND THEIR DISTRIBUTION IN CANADA[1]

By Margaret Newton and T. Johnson[2]

Abstract

Studies were made of the effect of temperature and light on the reactions to leaf rust *(Puccinia triticina* Erikss.) of the differential varieties of wheat used for the identification of physiologic races of this rust. With a large number of races both temperature and light were found to exercise a marked influence. The reactions of all varieties were not, however, influenced in the same direction. With lower temperature, Malakof and Democrat became increasingly susceptible, while Carina, Brevit, and Hussar became increasingly resistant. Webster and Mediterranean did not react consistently in either direction, and Loros was but little influenced by temperature. All of the differential varieties showed a more or less marked tendency to become increasingly resistant under conditions of short day length and weak light. In general, more pronounced changes in reaction were produced by variation of temperature than of light.

Surveys for the distribution of physiologic races of leaf rust in Canada were conducted annually since 1931 with the exception of the years 1932 and 1935. Forty-nine races were identified. Most of the prevailing physiologic races were found to be widely distributed throughout the country. Evidence was, however, secured that certain races were largely confined to certain areas. Races 1, 58, 76, and 81 were common for several years in Eastern Canada but were not encountered in the Prairie Provinces until 1940 when one collection of each of the three first-mentioned was made in that area. Races 11 and 53 were largely limited to British Columbia and the adjacent province of Alberta. Races of wheat leaf rust have undergone no marked change in respect to identity or relative prevalence in the last few years in Canada.

Introduction

Although studies on the specialization of wheat stem rust (*Puccinia graminis Tritici* Erikss. & Henn.) have been conducted in Canada since 1919, no attention was given to leaf rust (*Puccinia triticina* Erikss.) until 1931. There were two main reasons for this: (1) the much greater destructiveness of stem rust demanded that attention be first given to that rust, and (2) the fact that the varieties of common wheat grown in Western Canada until about 1930 were not highly susceptible to leaf rust. The introduction of highly susceptible varieties such as Kota and Ceres, resulted in a decided increase in the economic importance of this rust. The realization by plant breeders that the new wheat varieties that they were engaged in producing must possess resistance to leaf rust as well as to stem rust before they could be accepted as permanent substitutes for the older varieties, made it imperative that a study be made of the pathogenic characteristics of the leaf rust organism.

Mains and Jackson (7), in 1926, laid a foundation for studies on the specialization of the leaf rust organism by selecting a group of 11 wheat varieties

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by means of which they were able to demonstrate that the rust was specialized into many physiologic races. These eleven varieties were later reduced by Johnston and Mains (6) to eight, namely, Malakof (C.I. 4898), Carina (C.I. 3756), Brevit (C.I. 3778), Webster (C.I. 3780), Loros (C.I. 3779), Mediterranean (C.I. 3332), Hussar (C.I. 4843), and Democrat (C.I. 3384). These varieties, which have since gained general acceptance as differential hosts for leaf rust, were used in the identification of physiologic races of leaf rust by the writers in 1931. The variety Thew, used as a differential host by Waterhouse in Australia, was included with these varieties in the 1940 rust survey. As it proved susceptible to all collections of leaf rust made in that year, as well as to races 27, 28, 41, 65, 71, and 103, it is probably not a suitable variety to differentiate North American leaf rust races.

It soon became evident that the identification of these races was by no means a simple and easy task. The frequent presence of several physiologic races in a single collection of rust made it necessary to separate the races by propagating each culture of the rust from single uredial pustules or even single urediospores. A more serious difficulty, however, was the apparent instability of the host reaction of certain of the differential varieties. Frequently it was found that races identified without difficulty and put aside in storage would, when again tested after an interval of a few months, produce infection types considerably at variance with those produced when the race was originally identified. For example, races originally identified as race 13 or 20 were occasionally reidentified, under different environmental conditions, as race 31. In these instances the difficulty was obviously caused by instability of the reaction of the varieties Carina and Brevit. Similar difficulties were frequently experienced also with the reaction of the variety Hussar, the instability of which made it sometimes difficult to distinguish such races as 9 and 31 or 2, 15, and 34.

The apparent instability of the host reaction of these and certain other varieties led to a decision to investigate the effect of certain environmental conditions, particularly temperature and light, on the host reactions of the differential varieties used for the identification of physiologic races of leaf rust. The present paper attempts to give an account of this work as well as a summary of the physiologic race surveys thus far conducted in Canada. A summary of the physiologic races identified by the writers up to 1936 has already appeared in an earlier publication (8).

The Effect of Temperature and Light on the Reaction of Differential Varieties

Before the work here described was undertaken, it had been shown that environment was capable of influencing the host reaction of certain of the differential varieties. Mains and Jackson (7) stated that Hussar showed considerable variation in its reaction to some physiologic races, often being resistant in fall or winter but only moderately or slightly resistant in late spring. Waterhouse (12) noted that Carina and Hussar showed flecks in

the winter months and a "4" infection type in summer when infected with Australian race 1. Dodoff (1) stated that the reactions of Brevit were apparently influenced by temperature, and Gassner and Straib (2) showed that the varieties Malakoff, Norka, Democrat, and Mediterranean became increasingly susceptible with lower temperatures.

The Effect of Temperature

It is clear from the above statements that the environment is capable of affecting host reactions to a considerable degree. Only in the work of Gassner and Straib, however, had it been shown conclusively that a particular environmental factor (temperature) was responsible for a pronounced effect on host reaction. Gassner and Straib (2) showed that the reactions of Malakoff and Norka to race 14 varied from infection type "0" at 18.7° C. to types "3" and "4" at 6° C., and of Democrat from types "0" or "1" at 18.7° C. to types "3" and "4" at 16.6° C. and lower temperatures. As the varieties Webster and Similis produced the same reaction at 18.7° C., 16.6° C., 10.9° C., and 6.0° C., the response to low temperature was clearly not characteristic of all varieties resistant at ordinary temperatures.

That high temperatures can produce a comparable modification of host reaction was shown by Johnson and Newton (5) who found that at temperatures above 85° F. (29° C.) Little Club wheat became resistant to physiologic races to which it is susceptible at ordinary temperatures.

While the present work was in progress, further light was thrown by other investigators on the influence of temperature on the reactions of differential varieties. Roberts (9) stated that the effects of low temperature and low light intensity were similar—both having a tendency to increase resistance of varieties normally susceptible. Varieties normally resistant showed increased susceptibility with increased temperature and light intensity. Lacking facilities for control of temperature, she experienced difficulties in determining the relative importance of temperature and light in changing the varietal reactions.

Hassebrauk (3) showed that low temperatures (about 6° C.) generally increased susceptibility, though the reverse was true for some varieties. The varieties Carina and Brevit formed an exception in that they were more resistant at low temperatures.

Experiments conducted by the present writers on the influence of temperature on the reaction of the differential varieties to eight physiologic races of leaf rust are summarized in Table I. The tests were carried out in two different series of experiments, one conducted in April, 1936, the other in February, 1939. In both instances, the plants tested at the highest and lowest temperatures were kept in sections of the greenhouse with thermostatic temperature control. The plants tested at the medium temperature were kept in a section of the greenhouse in which temperature fluctuation was kept within narrow limits by manipulation of the steam pressure and the ventilators.

Thermographic records showed that fluctuations of temperature in this section were only slightly greater than in the sections with thermostatic temperature control.

TABLE I

INFECTION TYPES ON DIFFERENTIAL VARIETIES OF *Triticum vulgare* PRODUCED BY EIGHT PHYSIOLOGIC RACES OF *Puccinia triticina* AT THREE CONSTANT TEMPERATURES

Physiologic race	Temp., °F.	Date of expt.	Wheat variety: Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Race identified
	57	Feb.	4=	2−	1−	4−	4=	x−	x+	3+	No known race
9	64	1939	3+	2+	2	3+	3+	1±	2±	1−	Race 9
	69		3+	3+	3+	4−	3+	1±	3	1−	Race 20
			4	**1-2**	**1-2**	**4**	**4**	**0-1**	**1-2+**	**0-1**	
	57	Feb.	x+	x+	x+	x	3+	3+	x	3±	No known race
11	64	1939	0	x+	3	3=	3+	2	2−	1	No known race
	69		0	3	3+	x	3+	1	3	1−	Race 66
			0	**2+**	**3-4**	**1-2+**	**3-4**	**1-2**	**0-2**	**0-2**	
	60	Apr.	1−	1−	1	0	1−	3	2−	3	Race 15
15	65	1936	0	1−	1−	0	1−	3	2−	3	Race 15
	75		0	1−	3	1−	1−	3+	3	3+	Race 62
			0	**0**	**0-1**	**0**	**0-1**	**4**	**0-1**	**4**	
	60	Apr.	3	1+	1−	3+	3+	1−	3	x	Race 27
31	65	1936	3+	2+	2−	3	3+	1−	3	2−	Race 31
	75		3+	3+	3+	3+	3+	1−	3+	1−	Race 20
			4	**2**	**1-2**	**4**	**4**	**1-2**	**3-4**	**1-2**	
	57	Feb.	4	2±	1+	4−	4−	x	3	3+	No known race
41	64	1939	4−	3+	2++	4−	4−	1+	2++	3±	Race 41
	69		4−	3+	3+	4−	3+	3±	3+	3+	Race 77
			4	**2-3**	**2**	**4**	**4**	**2**	**2**	**3-4**	
	57	Feb.	3+	1	1±	1−	3	x−	x−	3+	No known race
44	64	1939	0	1±	2+	1−	3	x−	1−	3	Race 44
	69		0	1+	3	0	3	3	2+	3	Race 58
			0	**2**	**2**	**0-2**	**4**	**x**	**0-1**	**4**	
	60	Apr.	1−	3	3+	3	3+	x	2−	x	No known race
87	65	1936	1	3+	3+	3	3+	x	2	x	Race 87
	75		0	3+	3+	2−	3+	2−	3+	1+	No known race
			1	**4**	**4**	**3**	**4**	**x**	**2**	**x**	
	60	Apr.	1−	x−	2	3+	x	x	1	3+	No known race
88	65	1936	1	x	x	3	3+	x	1	3+	Race 88
	75		0	3+	3	1	3+	3+	3	3+	Race 12
			1	**2-3**	**2-3**	**3**	**4**	**x**	**1**	**4**	

Figures in bold-face represent the infection types recorded for type race in key prepared by H. B. Humphrey, C. O. Johnston, R. M. Caldwell, and L. E. Compton.

The plants in each series of experiments were exposed, as nearly as possible, to the same conditions of light. Inoculations of plants kept at all three temperatures were done on the same day. Light conditions at the different temperatures were not, however, absolutely identical because rust developed most rapidly at the highest and most slowly at the lowest temperature; the result was that the uredia, at the highest temperature developed about two days earlier than at the medium temperature and about seven days earlier than at the lowest temperature. Corresponding phases of rust development therefore took place under slightly different light conditions at the three temperatures.

The results of the experiments show clearly that there is a pronounced varietal response to temperature. The varieties Malakof and Democrat showed increasing susceptibility with lower temperature. The reaction of Webster to most of the races was not greatly influenced by temperature. To some races, however, this variety proved susceptible at 65° F. and resistant or moderately resistant at 75° F. The varieties Carina, Brevit, and Hussar showed increasing susceptibility with higher temperature. The behaviour of the variety Mediterranean was rather inconsistent, its susceptibility to certain races tending to increase with lower temperature, whereas to other races its susceptibility was greatest at the highest temperature. The reaction of the variety Loros did not seem to be affected by temperature.

The results of tests with Malakof and Democrat confirm the findings of Gassner and Straib (2). They obtained, however, no indication of any pronounced response in the reaction of Webster to temperature. The response to temperature of Carina and Brevit agrees with that reported by Hassebrauk (3) who stated that these two varieties were more resistant at low than at high temperatures. The temperature response of Hussar is in agreement with results reported by Mains and Jackson (7), Waterhouse (12), and Roberts (9), who agree that this variety tends to be more resistant in the winter months than in the summer.

An examination of Table I may give the impression that the increased susceptibility of Malakof at low temperatures applies only when this variety is infected with certain races. It should be pointed out, however, that the mean temperatures for the low temperature tests were lower in the experiments of February, 1939, (57° F.) than in those conducted in April, 1936 (60° F.). This difference in temperature, though slight, may possibly account for the difference in reaction of Malakof, which retained its resistance at all temperatures in the earlier series of experiments but became susceptible at the lowest temperature in the later experimental series. Controlled experiments and general experience alike indicate that greater fluctuations of temperature are required to influence the reaction of Malakof than the reactions of Carina, Brevit, and Hussar.

It is clear from the evidence presented in Table I that temperature must be taken into consideration in the identification of physiologic races. In several instances the same culture of rust appeared to be a different physiologic

race at each of the three temperatures employed. A culture originally identified as race 31 appeared to be race 27 at 60° F., race 31 at 65° F., and race 20 at 75° F.* In view of such results it seems probable that pathogenically similar strains of rust have not infrequently been described as different physiologic races merely because the environmental conditions differed in the places at which identifications were made or in the seasons in which the work was performed.

The Effect of Light

Most of the evidence relative to the effect of light on host reaction to leaf rust is somewhat indirect in so far as only rarely has the effect of light been clearly separated from that of temperature. It is highly probable that variation in light (intensity of light and duration of day length) played a part in the production of the pronounced differences in varietal reaction in winter and summer noted by several investigators. Mains and Jackson (7) observed that Hussar was more resistant to certain physiologic races in winter than in summer. Waterhouse (12) reported that the varieties Hope, Iumillo, Hussar, and Carina, which were susceptible to Australian race 1 in summer, showed an indeterminate "x" reaction in winter and that the variety Thew, also susceptible in the summer months, proved highly resistant in winter. Roberts (9) found in the reactions of the variety Webster to race 73 a seasonal variation that appeared to be related to intensity of light. Tests conducted by her at the beginning, at the middle, and at the end of October, produced reactions of "4", "3+", and "2+" respectively. Her next test, which was made in February, produced the "x" reaction whereas the type "4" reaction was again produced in March and in subsequent tests in June, July, and August.

Recently, a study was made by Hassebrauk (3) of the effect of light on the reaction of the differential varieties to several races of leaf rust. In his study, the plants, following infection, were subjected to two different daily periods of light—one set of plants being exposed to normal daylight, another to only three or four hours of light per day. In general, reduction of light had either no effect on host reaction or tended to increase resistance slightly. The varieties Carina and Brevit proved exceptional in that their responses to light varied according to the races to which they were tested. To certain races they proved slightly more resistant under conditions of reduced light whereas to other races they were decidedly more susceptible in reduced light.

Still more recently Hassebrauk (4) has studied the effect of darkness during various portions of the incubation period on varietal reaction to leaf rust. Varieties moderately resistant under normal light conditions showed, when kept in darkness from the second to the fourth day of the incubation period, a definite increase of susceptibility. Darkness from the fourth to the sixth day tended to retard pustule development without greatly influencing reaction.

** Chester and Jamison (Phytopath. 29 : 962-967. 1939) have recorded somewhat similar difficulties in distinguishing between certain physiologic races. They regard races 9, 13, and 19 as environmental variants of the same race.*

Darkness during the later portions of the incubation period generally increased resistance beyond that of control plants kept in normal light.

The possibility that seasonal variation in light intensity and daily duration of light might produce an important effect on the reaction to leaf rust of the differential wheat varieties led the writers, in the late winter and spring of 1936, to conduct experiments on the effect of light on host reaction. These experiments were carried out in two greenhouse sections with thermostatic temperature control; one section was maintained at a temperature of 60° F., the other at a temperature of 75° F. Three series of experiments were conducted: one in February (February 1 to 19), one in March (February 27 to March 12), and one in April (April 4 to April 17). These experiments are summarized in Table II, which shows the average reaction of six wheat varieties under conditions of approximately constant temperature but progressively increasing day length and light intensity. The increase in intensity of light in the greenhouse was considerably greater than out-of-doors owing to the presence on the glass, in February, of a coating of frost which had a noticeable shading effect.

In general, there was a tendency for susceptibility to increase somewhat as the day grew longer and light became more intense. This tendency was shown to a greater or less degree by all the varieties tested. Of the seven physiologic races used, all reflected the influence of increased light by their infection types on one variety or another. Several instances are recorded in Table II of a reaction, normally susceptible, being shifted towards resistance by low light intensity. Carina and Webster which are normally susceptible to races 10 and 87 tended, in February, to produce an indeterminate "x" reaction at 60° F., and Hussar, which normally is susceptible to race 52, showed an indeterminate reaction in March and a resistant reaction in February. Increased susceptibility with decreased light, such as reported by Hassebrauk (3), was not noted except perhaps in the reaction of Brevit to race 89 at 60° F.

Although light apparently induced some variation in normally susceptible reactions, the ones that responded most frequently were the reactions representing moderate or slight resistance, such as those described in the analytical key as "1" to "2", "2" to "3", or "x". Reactions ordinarily classified as "1" to "2" varied in some instances from "1" to "x", and reactions ordinarily indeterminate in some cases varied from 1 to 3. Evidently the reactions representing moderate or slight resistance are less stable than those representing high susceptibility or high resistance.

The fact that all tests were conducted at two temperatures makes possible a comparison of the relative influence on host reaction of light and temperature. In most instances, the maximum variation attributable to temperature was greater than that attributable to light. In some cases in which light showed no influence on reaction temperature exercised a profound effect. An example of such a temperature effect is shown in the reactions of Carina and Brevit to race 31, which were recorded as "1−" to "1+" at 60° F. and "3" to "3+" at 75° F.

TABLE II

INFECTION TYPES ON DIFFERENTIAL VARIETIES OF *Triticum vulgare* PRODUCED BY SEVEN PHYSIOLOGIC RACES OF *Puccinia triticina* AT A LOW, MEDIUM, AND HIGH LIGHT INTENSITY AND CONSTANT TEMPERATURES OF 60° F. AND 75° F.

Physiologic race	Temp., °F.	Wheat variety																					
		Carina			Brevit			Webster			Similis			Mediterranean			Hussar			Democrat		
		Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April
10	60° / 75°	x / 3	3− / 3	3 / 3+	3− / 3	3 / 3+	3+ / 3+	x− / 1+	x− / x	3+ / 3−	1+ / 1+	x− / x+	x / x+	x / ;	x / ;	x / 1−	1− / 3+	x− / 3+	x / 3+	2− / ;	1− / 1−	x / ;
		4			**4**			**4**						**1-2**			**1-2**			**1-2**		
31	60° / 75°	1 / 3+	1+ / 3	1+ / 3+	1− / 3	1− / 3+	1− / 3+	3+ / 3+	3+ / 3+	3+ / 3+	3+ / 3+	3+ / 3+	3+ / 3+	1− / ;	1− / ;	1− / 1−	3− / 3+	3− / 3+	3 / 3+	1+ / ;	x− / ;	x / 1−
		2			**1-2**			**4**						**1-2**			**3-4**			**1-2**		
52	60° / 75°	; / ;	; / 1−	; / 1−	; / 1	; / x=	1− / 2+	; / ;	; / ;	; / ;	; / ;	; / ;	; / ;	3+ / 3+	3 / 3+	3+ / 3+	1 / 3−	x / 3+	3 / 3	3+ / 3+	3 / 3+	3 / 3+
		0			**1**			**0**						**4**			**3**			**4**		
85	60° / 75°	— / —	3 / 3−	3+ / 3+	3+ / 3	3+ / 3+	3+ / 3+	— / —	x− / x−	3+ / 2+	2 / —	x− / 2−	3+ / 2+	— / 3+	3+ / 3+	3+ / 3+	— / 3	x− / 3+	x / 3+	— / 3+	3+ / 3+	3+ / 3+
		4			**4**			**2-3**						**4**			**3**			**4**		
87	60° / 75°	2+ / 3	x+ / 3+	3 / 3+	3 / 3	3 / 3+	3+ / 3+	x / 1+	x− / 2−	3 / 2−	x− / 1+	x− / 2−	3+ / x+	x / ;	x+ / 2+	x / 2−	2− / 3−	x / 3+	2− / 3+	x / ;	x / 2−	x+ / 1
		4			**4**			**3**						**x**			**2**			**x**		
88	60° / 75°	; / —	; / 3+	x− / 3+	2− / 2−	2− / 3+	2 / 3	; / —	; / 1−	3+ / 1−	; / 1−	x− / 1−	3+ / 2−	— / —	x / 3+	x+ / 3+	1− / —	1− / 3+	1 / 3	3+ / 3+	3+ / 3+	3+ / 3+
		2-3			**2-3**			**3**						**x**			**1**			**4**		
89	60° / 75°	3 / 3−	— / 3	3− / 3	3+ / —	x− / 3+	x / 3+	1 / 3+	x / 1+	3 / 3+	1 / 3+	x / 2−	3+ / 3+	3+ / 3+	3 / 3+	3+ / 3+	3+ / 3+	3 / 3+	3 / 3+	3+ / 3+	3+ / 3+	3+ / 3+
		x			**x**			**x**						**4**			**4**			**4**		

Figures in bold-face represent the infection types recorded for type race in key prepared by H. B. Humphrey, C. O. Johnston, R. M. Caldwell, and L. E. Compton.

Distribution of Physiologic Races in Canada

The annual surveys of the distribution of physiologic races of leaf rust in Canada are summarized in Table III. These surveys have been carried out since 1931 with the exception of two years—1932 and 1935. Owing to the fact that all but one of the surveys (1934) covered practically the whole of Canada, it is possible to arrive at some conclusion as to the distribution of physiologic races in different parts of the country, and their persistence in those parts from year to year. In tabulating the results, the country was divided into four regions of which the agricultural areas are to a certain extent separated by natural barriers: first, the Maritime Provinces which are partially isolated from the remainder of Eastern Canada; second, the provinces of Ontario and Quebec; third, the Prairie Provinces of Manitoba, Saskatchewan, and Alberta, which are isolated from the farming areas of Ontario by great spaces of lake and forest; and fourth, British Columbia which is separated from the prairie region by the Rocky Mountains.

The surveys have shown that certain races are largely confined to certain regions and may be found to occur there annually whereas other races are widely distributed throughout the whole country. Races largely confined to Eastern Canada (Ontario, Quebec, and the Maritime Provinces) include races 1 58, 76, and 81. These have been collected year after year in the eastern provinces but have not been found in the prairie region of Western Canada except in 1940 when one collection of each of the three first-mentioned races was made in that area. The last three (races 58, 76, and 81) bear a rather close resemblance to each other pathogenically, and had perhaps better be regarded as biotypes of the same race rather than as three separate races.

There appear to be no well authenticated examples of races localized in the Prairie Provinces, but there is evidence of the localization of certain races in British Columbia. Race 11 had been collected 20 times since the surveys were begun, 17 times from British Columbia, twice from the adjacent province of Alberta, and once from the Maritime Provinces. Race 53 has been collected 11 times, 7 times in British Columbia, twice in Alberta, and twice in Manitoba. The occasional occurrence of the same races in British Columbia and in the adjoining province of Alberta suggests a limited amount of rust spread eastward either across the western mountain ranges from the coastal region, or northward from the Northwestern States. As the mountains seem to act as a barrier to the westward spread of rust, the latter assumption appears to be the more probable. At least, several races (races 5, 31, 44, 52) found commonly in the prairie region have never been collected in British Columbia.

Most of the predominant races, however, are not limited to any one region of the country. Races 2, 5, 9, 15, 31, 32, 34, 52, and 89 have been collected year after year both in Eastern Canada and the Prairie Provinces. The fact that these races were collected year after year would seem to indicate that,

TABLE III

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *P. triticina* FROM 1931 TO 1940.
(FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA.)

Physiologic race	Maritime Provinces							Ontario and Quebec							Prairie Provinces								British Columbia							Total
	1931	1933	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940	1931	1933	1934	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940	
1				1	1	5	6			2	2		2									1*c*			1					21
2	1				4	4		2					3	2	1	1		1		1	7	4						1		32
3						1								2						1		2								6
5					1					2	2	1	5	1		1		2	7	5	5	2								34
6														1					2		1									4
9					2	1	1			1	1	4	1	1			1		3	7	6	3					1			33
10																							1							1
11						1														1*a*		1*a*				3	3	6	5	20
13															3					4										7
14															1								1		1					3
15		1		4	8	6	5	1		4	3	3	3	3	2	3	1		2	4	6	6	5	1		5		2	5	83
19																			3											3
20	2							1							1															4
27																		1	1	1										3
28				1															1		1									3
29					1	3						2	5						4		3	2								20
30		1													1	6				2			1							11
31										1		1	1	3	14	3	1		1	7	9	2								43
32		1		1	4		3					1		1		2				1		3							3	20
34					2	2	2			2	1		1	1					1	1	3									16
35			1													1	1													3
37													1																	1
39													1																	1
41												1																		1
42								1																						1
44		1		1							1			2	1	1	3		2			1								13
50										1	1																			2

NOTE: *(a) = Collected in the province of Alberta; (b) = Collected in the province of Manitoba; (c) = Collected just north of Alberta boundary.*

TABLE III—*Concluded*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *P. triticina* FROM 1931 TO 1940.
(FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA.)
—*Concluded*

Physiologic race	Maritime Provinces							Ontario and Quebec							Prairie Provinces								British Columbia							Total
	1931	1933	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940	1931	1933	1934	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940	
52	1					1					1		2		2	1			4		9	1								22
53															1*a*	1*a*					1	1	3			1	1	2		11
56	3	2													2	2								1						10
58						8	1					1	3	2								1*c*								16
62			1																											1
64																			1											1
65										1									3	1										5
66															1								1							2
72																						1								1
76			6	3	9	4	9			2	1	4	5	4								1*b*				2				50
77															1															1
80										3		1							1											5
81				4							3	3		1															1	12
83																						1								1
85																1														1
87									1														2							3
88																	2													2
89			1						1	2		2	1	3		2		1	4	1	10	2			1			1	1	33
90																	1													1
101						1								2				1												4
103						1																								1
104							1					1		2						1	1									6
	7	6	9	15	32	38	28	5	3	20	17	25	36	28	31	25	10	6	40	38	62	35	14	2	3	11	5	12	15	578

NOTE: *(a) = Collected in the province of Alberta; (b) = Collected in the province of Manitoba; (c) = Collected just north of Alberta boundary.*

in respect to identity and relative prevalence, no radical change has taken place in this rust during the past few years.

Discussion

The difficulties involved in the identification of physiologic races of leaf rust appear to result chiefly from two causes: first, the high degree of specialization of the leaf rust organism and, second, the sensitivity of the reactions of certain of the differential wheat varieties to environmental influences. Because of the high degree of specialization, numerous pathogenically different strains of the rust are encountered each year, and, as the variations in pathogenicity are often slight, the investigator is frequently faced with the problem of deciding whether or not two or more pathogenically similar but not quite identical cultures should be regarded as one and the same race. As pointed out by Johnston and Mains (6) and Scheibe (10, 11), such pathogenic variants could almost certainly be distinguished clearly from each other by the addition of new varieties to the differential hosts now in use. Such a course would, however, in all probability have little practical value and would increase considerably the work involved.

Recognition of the second difficulty, namely, the responsiveness of the reactions of certain differential varieties to variations in temperature and light, has led investigators (2, 3, 9) to emphasize the importance of conducting such studies, as far as possible, under conditions of constant temperature and light. Most of the identification work, however, must perforce be carried out in greenhouses in which these factors can be controlled only to a limited extent. It is obvious, therefore, that a knowledge of the ways in which light and temperature influence rust reactions is of great importance. With such knowledge, allowance could be made for such influences at different seasons of the year. The experiments with temperature and light reported in this paper were designed to furnish such information. The results obtained, together with those previously presented by other investigators, make it possible to gauge to some extent the modification of host reaction induced by variation in these two important factors of the environment.

It seems clear from the experiments here reported and those of other investigators that the reactions of the various differential varieties respond differently to temperature. Malakof, Norka, and Democrat tend to become increasingly susceptible with lower temperature. Carina, Brevit, and Hussar, on the other hand, become increasingly resistant with lower temperature. All of the differential varieties show a more or less marked tendency to become increasingly resistant under conditions of short day length and low light intensity. These facts, judiciously applied, should be of considerable assistance in the work of determining physiologic races by means of the differential varieties now in use. It would, of course, be more satisfactory to use as hosts varieties whose rust reactions showed little or no response to environmental conditions. The elimination, however, of the more reactive varieties, such as Carina, Brevit, and Hussar, and the substitution, as suggested by Hassebrauk (3) of other

more reliable varieties for them, if such could be found, might have the advantage of simplifying the work of identification, but, on the other hand, might have the disadvantage of making it difficult to relate the physiologic races identified by means of the new hosts with those identified in previous years by means of the discarded hosts. This difficulty would particularly apply if races identified in earlier years but now no longer available for study should reappear. With new differential hosts such races might be reidentified as wholly new races. In other words, a change of differential varieties might interfere with one of the important functions of physiologic-race surveys, namely, that of detecting the presence of new physiologic races.

The surveys for physiologic races carried out since 1931 indicate that races of wheat leaf rust have undergone no marked change in respect to identity or relative prevalence during this period in Canada, unless the appearance of race 76, in 1936, in the eastern provinces be regarded as such. As the surveys, prior to 1936, for leaf rust in Eastern Canada were rather inadequate, it is quite possible that this race was also present there in earlier years.

The reason for the localization of one or more races in certain areas is not altogether clear. Presumably a race, such as race 76, may overwinter in Eastern Canada or in some region to the south from which it may spread northward annually. The overwintering of certain races somewhere along the Pacific Coast and the obstruction to their eastward spread offered by the Rocky Mountains may perhaps account for the localization of those races in that region.

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STUDIES OF WATERFOWL IN BRITISH COLUMBIA

GREATER SCAUP DUCK, LESSER SCAUP DUCK[1]

BY J. A. MUNRO[2]

Abstract

Nyroca marila is an abundant migrant through British Columbia and large numbers winter in the coast region. The sex ratio in winter flocks is predominantly male. *Chara* was the chief food eaten by 57 specimens from Okanagan Lake, miscellaneous vegetable matter was second, and molluscs third in importance. Food items, listed in order of importance, on coast streams and lakes were: vegetable matter, molluscs, salmon eggs, salmon flesh, and, on salt water: gastropods, sea lettuce (*Ulva* sp.), crustaceans, and herring eggs. *N. affinis* nests commonly in parts of the dry interior and elsewhere in the province is a migrant and scarce winter visitant. Sex ratio is predominantly male. Courtship continues through April and May; laying commences in June and late clutches are found in August. Females defend their young vigorously and a habit of combining broods has a probable survival value. Males raft on certain lakes in July and go into eclipse as flight feathers are shed. These populations include yearling and post-breeding females and later, adolescents. The former moult at this time. Adults migrate early and those remaining are largely young of the year. Amphipods are the chief food of all age groups on the nesting ground; aquatic insects and seeds of aquatic plants are also important. Both species of scaup ducks are economically important as food and for sport in the interior but less so on the coast where, because of a different diet, their flesh is less palatable. It was not determined whether the consumption of salmon eggs and herring eggs is of economic significance. Elsewhere than on the coast scaup ducks are related to other interests only to the limited extent to which they are food competitors of trout and other commercially valuable fishes.

Introduction

The present paper is submitted as a contribution to the life histories of the scaup ducks as observed in British Columbia. In the case of the Greater Scaup Duck, *Nyroca marila* (Linn.), known only as a migrant and winter visitant, the data concern chiefly its winter range, numerical status, and food habits. Concerning the Lesser Scaup Duck, *Nyroca affinis* (Eyton), which nests commonly in parts of the province, more detailed information, particularly about behaviour of summer populations, is presented.

A study of the migratory movements of the scaup ducks is complicated by the fact that the two species resemble each other closely and much observational data cannot be accepted. Although they may be separated readily enough in the hand, it is usually more difficult and sometimes impossible to

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do so in the field. The smaller size of *N. affinis*, the smaller amount of white on its wing and the specific characteristic of greenish or purplish head reflections in adult males are relative characters apparent only under favourable conditions. Nevertheless from many observations in the field and from the more satisfactory evidence afforded by specimens examined closely, it seems clear that the winter population in British Columbia is predominantly the species, *N. marila*, and the nesting population is exclusively *N. affinis*.

Field observations and records of stomach analyses covering the period 1911 to 1940 form the basis of this paper. As in earlier contributions of a similar nature, the food studies were largely a joint undertaking by Dr. W. A. Clemens, then Director of the Pacific Biological Station, and the author. The methods of laboratory examination described by Munro and Clemens (13) were followed.

Greater Scaup Duck

Distribution on the Pacific Coast

There are no records of the Greater Scaup Duck nesting in British Columbia nor in the United States south of this province. Except for the probable occurrence in summer of non-breeding individuals, its status is that of a winter resident. The breeding grounds lie well to the north and east of British Columbia. In winter it is abundant on the coast of southeastern Alaska, British Columbia, and Washington. It is not recorded by Jewett and Gabrielson (6) from the Portland area, Oregon, and once only from Netarts Bay (17). Further investigation in Oregon may prove the species to be more plentiful than the available records indicate since it is a common midwinter visitant to the coast of northern California. According to James Moffitt (personal letter, January 30, 1940), as many as 6000 have been observed at Tomales Bay (January 20, 1929) and 4000 on Humboldt Bay (January 19, 1932).

Spring Migration

In parts of the interior of British Columbia, the Greater Scaup Duck is a regular spring migrant, arriving somewhat earlier than the Lesser Scaup Duck and remaining on some of the larger lakes where food is plentiful for a month or six weeks. On the open water of the Thompson River, small flocks have been reported in February (73 at Kamloops, February 10, 1940). In the Okanagan first arrivals are usually observed in March, for example, 32 at Okanagan Landing, March 28, 1932. By the latter part of April, the migration of *N. marila* is nearly over whereas that of the *N. affinis* is at its height. Thus on a series of small ponds near Kamloops (April 25, 1939) only 35 in a total of 500 scaup ducks were identified as *N. marila*.

The greater part of the winter population, however, goes slowly up the coast, their movements governed largely by the movements of Pacific herring which at this time (late February to early April) are spawning. During the height of the spawning runs the largest number of greater scaups are present and the greatest concentrations take place. The following estimates indicate the usual size of these flocks: 2000 at Departure Bay, March 3, 1928; at

Nanoose Bay, 4500 on March 11, 1928; 4000 on March 10, 1931; 3000 on March 26, 1936; 8000 on March 21, 1940; 1500 at Qualicum Beach, March 15, 1939. Late dates for spring migrants are: Victoria, May 14, 1904; Clayoquot, May 22, 1931; Masset Inlet, Queen Charlotte Islands, May 26, 1920. The last refers to a flock of 30 that were chiefly yearling males.

Autumn Migration

The difficulty of distinguishing between the species of scaup ducks has been pointed out. This is particularly the case in the autumn when there is the maximum association of the two species and the birds are so wary that close observation usually is impossible. At this time also there is difficulty in sight sex determination as some adult males are in eclipse and young males show white cheek patches similar to those worn by the adult female.

In the Okanagan Valley the first fall migrants appear in early October (specimens taken at Swan Lake, October 6, 1933, and October 4, 1934).

The fall population in the Okanagan reaches its maximum usually in early November and the majority disappear with the first freeze up, which normally occurs toward the end of that month. Subsequently, small flocks remain for a time on such of the larger lakes as have remained open but it has been unusual for the past 15 years for any to be present after late December.

Along the southern part of the coast region early arrivals are reported in September, for example at Comox, September 11, 1922, 25 were counted (Canadian National Museum record). The first large flocks usually are seen in early October, for example, 200 at Oak Bay, October 13, 1912. The migration continues through October and reaches its peak in November, after which the numbers decline. There remains a winter population (December-February) fluctuating in numbers and, in part, moving from salt water to fresh water feeding grounds.

Winter Populations

In earlier years several hundred greater scaups usually wintered on Okanagan Lake near its northern end and smaller numbers near the south end of this lake. In both places are shallow feeding grounds that completely freeze over only in exceptionally cold weather. Thus in January, 1918, a total of 150 and, in February, a total of 50 was counted whereas in March the number increased, by the addition of migrants, to 400. In 1919, a raft of approximately 200, of which about 70% were males, remained in the vicinity for the same period. They were last seen in such numbers in February, 1921, and since that time the records refer to flocks numbering up to 50 observed usually no later than December, for example, 25 on December 31, 1930; 2 on December 14, 1931; 50 on November 14, 1932; 12 on December 30, 1932; 36 on December 23, 1938.

In the mild winter of 1939–40, a flock of 25 wintered on the Thompson River near Kamloops but, in general, winter populations in British Columbia are now chiefly restricted to the coast region, where flocks up to 500, or more,

frequent suitable waters. In some localities, particularly the several bird sanctuaries comprising sheltered tidal waters on Vancouver Island, they become quite fearless of man and may be studied at close range, thus simplifying the matter of identification. Normal-sized flocks are indicated by the following counts: 149 at Nanaimo Harbour, January 7, 1939; 150 at Departure Bay, January 26, 1935; 400 at Ross Bay, February 16, 1940; 300 at Shoal Bay, February 16, 1940.

Sex Ratio of Greater Scaup Duck

In all flocks of Greater Scaup Ducks examined in winter, an excess of males over females has been observed. In large flocks examined at a distance it has not usually been possible to make exact counts but estimates suggest a proportion of six or seven males to one female. Where winter flocks were examined at close range and precise determination made, it was observed that the sex ratio varied, for example: 61 ♂ and 7 ♀ at Esquimalt Lagoon, February 26, 1940; 60 ♂ and 40 ♀ at Departure Bay, March 20, 1940.

Food and Behaviour of Greater Scaup Duck in the Interior of British Columbia

When present on one of the large lakes in the interior of British Columbia a flock of Greater Scaup Ducks may form part of a raft of diving ducks that have gathered on a rich feeding ground, such as a bed of *Potamogeton* or *Chara*. In these associations of diving ducks, which nearly always include American Coot, *Fulica americana*, and Baldpate, *Mareca americana*, the scaups usually form a unit; less often they are scattered through the raft. The waterfowl making up these large flocks invariably are wary. They stay well out from shore and, if not disturbed, may remain, feeding and resting, in the same locality during the greater part of the day.

When associated in small flocks, the greater scaups usually are less nervous and often feed close to shore. At such times all the members of a small flock up to a dozen or so sometimes submerge almost simultaneously, a habit that may be used to advantage by hunters on shore.

After the hunting season, certain small flocks in the vicinity of settlements become quite tame. Thus at Okanagan Landing in the winter of 1920–21, a small number congregated about the wharf each day when the steamer, then in service on the lake, tied up. These birds in response to a whistled call would swim quickly towards the wharf and feed upon scraps thrown to them within a few yards of interested spectators.

The leaves of *Potamogeton* and branchlets and oospores of *Chara* are prominent items of diet. It is known that *Chara* may suddenly become scarce or disappear from places where it has been abundant and not become re-established for several years. Possibly this is a factor influencing the local distribution and abundance of the Greater Scaup Duck and other species of diving ducks in the interior.

Quite recently a changed environmental condition has been brought about in a part of Okanagan Lake through the rapid replacement of *Chara*, the most valuable food in the lake, by the less desirable water weed, *Elodea canadensis*. Okanagan is a deep lake with only limited areas of feeding grounds and over much of these areas *Chara* was dominant until 1937. It grew profusely and in places the broken residue piled up on the shore to a depth of 12 in. or more. About 1936, *Elodea* appeared in quantity; at present (1940) it is dominant over much of the feeding ground near Okanagan Landing and drifted shore accumulations of aquatic vegetation is composed almost exclusively of this plant.

Food Summaries

In the section following is given a summary of the food eaten by the Greater Scaup Duck in the Okanagan District, British Columbia, as indicated by the examination of the contents of 79 stomachs from two localities, namely, Okanagan Lake and Swan Lake.

The Okanagan Lake material, collected in the winter chiefly from 1912 to 1915, is of some historic interest as it reflects a condition that has passed away. It has been mentioned that in the period stated and for some years later the Greater Scaup Duck wintered regularly on Okanagan Lake and that since that time it has practically disappeared as a member of the midwinter bird life. The ecological modification of the area has been referred to.

In the following summary the number of specimens examined is indicated by the figure following each of the months represented.

Okanagan Lake—November, 3; December, 9; January, 28; February, 26; March, 2.

Unidentified fishes. A trace of fish remains was a minor item in one and represented 2% of the contents of another stomach.

Crustaceans. The single occurrence of an amphipod represented 1% of the stomach contents; an isopod was a minor item in another specimen.

Caddis. In eight analyses caddis was found in four specimens, constituting less than 1%, and 2 to 12% of the food eaten, in the remainder.

Other insects. Fragments of Coleoptera bodies constituted 10% of the material in one, and less than 1% in three, stomachs. Chironomid larvae had been eaten by one specimen and represented 25% of the stomach contents. Corixids, Zygoptera, and Hymenoptera (ants) were each represented once as minor items.

Chara sp. Branchlets and oospores of *Chara* were found in all but five of the fifty-eight stomachs and were the chief or exclusive items in 43 specimens.

Sagittaria sp. Ground up fragments of *Sagittaria* tubers were the exclusive item in four, and represented 50 and 75% of the contents of two other stomachs.

Potamogeton sp. Stems, winter buds, and rootlets occurred in 10 stomachs, in six specimens representing 65 to 100% of the total contents; seeds of several species occurred in nine stomachs.

Miscellaneous vegetation. To this category are referred 23 records of plant fragments including *Zanichellia palustris*, *Rupia maritima*, and unidentified species. Material of this nature was the chief item in six specimens.

Miscellaneous seeds. Seeds of the following plants were represented the number of times indicated: *Scirpus americanus*, 7; *Polygonum* sp., 3; *Polygonum amphibium*, 2; *Cornus* sp., 3; *Najas flexilis*, 2; *Amaranthus* sp., 1; *Batrachium* sp., 1; *Hydrocotyle* sp., 1; *Triticum* sp., 1.

Gastropods. Snails, including *Limnaea palustris*, *Limnaea vahli*, *Planorbis* sp., and *Valvata virens*, represented respectively 14, 25, 42, and 98% of the contents of four and were present as minor items (8 to less than 1%) in 11 other stomachs.

Pelecypods. One specimen taken in March contained a whole specimen of *Anodonta* sp. measuring 45 mm. Other species represented were: *Pisidium abditum* and *Sphaerium occidental*, which occurred 15 times; they usually represented a small proportion of the contents but in one case constituted 46, and in another 53% of the total contents.

Summary.—*Chara* is the most important food both in frequency of occurrence and average percentage volume; a decided preference for this food is indicated. *Potamogeton* and other vegetable matter are second and mollusca third in total percentage volume; insects are of slight importance in the winter months.

Swan Lake, Okanagan,—November, 7; December, 4.

This is a shallow marshy lake three and one-half miles long which freezes over in winter. The specimens were taken here in the autumn of 1939, a year in which the usually abundant *Chara* was scarce.

Aquatic insects.

A specimen taken in December had eaten over 100 chironomid larvae (bloodworms); corixids and dragonfly nymphs each occurred twice.

Molluscs. Small specimens of *Planorbis* were present in one, and fragmentary mollusc shells in a second, stomach.

Seeds. In several specimens a few seeds of *Ceratophyllum demersum* were the only item of food; other species represented by seeds were: *Potamogeton pectinatus*, *Scirpus americanus*, *Zanichellia palustris*, and *Carex* sp.

Miscellaneous vegetation. Comminuted vegetable matter was identified in one case as *Potamogeton pectinatus*, in another as *Ceratophyllum demersum*.

Summary.—Seeds are the chief item in frequency of occurrence and average percentage volume. Miscellaneous vegetable matter is second and miscellaneous aquatic insects third in importance.

Food and Behaviour of Greater Scaup Duck in the Coast Region of British Columbia

Salmon ova, which become available soon after the greater scaups have arrived on the southern British Columbia coast, are a seasonal food of some importance. Spring salmon, *Oncorhynchus tschawytscha*, begin to ascend the coast streams with the first fall freshets; then come coho salmon, *Oncorhynchus kisutch*, sockeye salmon, *Oncorhynchus nerka*, pink salmon, *Oncorhynchus gorbuscha*, and chum salmon, *Oncorhynchus keta*. The spawning season of the five species covers the period from October to December inclusive. At this time, on the part of the ducks and gulls that feed upon salmon eggs, there is great activity and constant movements from the ocean to the streams.

The Greater Scaup Ducks may visit a spawning stream at any time of the day and remain for only a short time or they may arrive shortly after dawn and remain until dusk. No evidence of night feeding has been obtained. All appear to spend the night on the sea or on lakes drained by the spawning streams. The number visiting the streams varies from week to week, more being present during the actual spawning periods, and for a few days afterward, than at other times. In a few days after a heavy spawning, drifting eggs are easily obtainable from the bottom of the stream. The eggs taken appear to be exclusively those that have not been covered with gravel during the spawning or that have been dislodged during subsequent spawnings by freshets or some other agency. No evidence that the Greater Scaup Duck extracts eggs out of the redds has been obtained.

The rotted flesh of dead salmon that have stranded along the spawning stream also is eaten at this season. The gullets of two greater scaups, shot on the Goldstream river in November, 1938, were filled with this semi-liquid material.

Perhaps the most important seasonal food is the ova of Pacific herring, *Clupea pallasii*, and when the spawning season is at its height in March and early April large concentrations of greater scaups and other diving ducks take place over the spawned areas and on adjacent waters. Usually the different species are more or less segregated, forming an association of flocks rather than of individuals, but after being flushed several times these units tend to break up and the different species to mix indiscriminately. For example, at Nanoose Bay (March 12, 1934) several thousand Greater Scaup Ducks in a single flock were close to, but not intermingled with, smaller flocks of White-winged Scoters, *Melanitta deglandi*, and Surf Scoters, *Melanitta perspicillata*. After these had been disturbed by a passing launch, no further segregation was observed during that day. Upon another occasion at the same place (March 26, 1936) approximately 3000 Greater Scaup Ducks were associated with a smaller number of White-winged Scoters and Surf Scoters but no segregation of species was apparent. In these large rafts of ducks the greater scaup is invariably the most wary of the species assembled and when approached by boat all will take wing almost simultaneously, several

minutes before ducks of other species show signs of alarm. The relative ease with which the greater scaups leave the water, their tendency to attain altitude immediately, the compact nature of the flock, and the mobility of the birds in the air is in marked contrast to the less agile movements of the other diving ducks as they rise.

Herring deposit their eggs on various kinds of algae or on eel-grass, *Zostera marina*, in relatively shallow areas close to shore. Those that do not adhere to these growths sink to the bottom and accumulate amongst the vegetation or in depressions on the sea bottom. These deposits of unattached eggs are considered to be the chief source of supply for the greater scaup and other diving ducks.

Only one specimen of greater scaup shot on the spawning grounds was available for examination but it has been observed that their method of obtaining this food is similar to that of White-winged Scoters, Surf Scoters, American Golden-eye, *Glaucionetta clangula americana*, Buffle-head, *Charitonetta albeola*, and Old Squaw, *Clangula hyemalis*. All obtain the eggs by diving; none have been observed on the spawning grounds when the eggs are exposed at low tide. The stomachs of these ducks contained usually a mixture of herring eggs and sand (indicating that the material had been scooped from the sea bottom) and infrequently a mixture of herring eggs and vegetation.

Salmon eggs and herring eggs are seasonal foods only; the staple item of diet from salt water consists of various small molluscs that are obtained by diving close to shore. Crustaceans obtained in similar situations are a food of less importance. Sea lettuce, *Ulva* sp., is eaten extensively and small flocks of greater scaups frequently have been watched as they dived for this food. Thus at Departure Bay (February 18, 1934) four adult males were observed from a concealed position almost directly above them. From this vantage point all objects on the sea bottom and each movement of the active ducks was clearly visible. Glistening air bubbles adhered to their white flank feathers that lightly encompassed the wings as the birds swam down through the clear water. They remained below 30 sec., more or less, then rose buoyantly as if suddenly released by a spring. Immediately on reaching the surface, the sea lettuce, which hung pendent from their bills, was shaken from side to side with vigorous movements of the head. This head movement was repeated numerous times until the sea lettuce was swallowed.

In the vicinity of the coast cities, greater scaups frequent the waters at the outlets of sewers and return to these places year after year. They are constant visitors also to the boat slips at city wharves where, in company with several species of gulls, they feed upon scraps thrown from the ship galleys. In some harbours there invariably is a flock in regular attendance until the spawning of herring in the spring attracts them elsewhere.

In waters where shooting is not permitted they show varying degrees of fearlessness of man. An extreme example is reported from Victoria where in a sheltered lagoon a fisherman captured by hand and banded a female that,

on hearing a certain call, was accustomed to swim up to him and be fed. This banded bird returned to the same locality for four successive winters (1937–1940) and each year was handled by the fisherman.

During the months of February and March there is a certain amount of courtship activity that increases as the season advances. Birds that have been feeding suddenly commence to bow, thrusting the head forward with neck arched until the tip of the bill touches the surface, then raising the head until the bill is in a vertical position or pointing slightly backward. Or the action may be less vigorous, the bill dropped gently to the surface, then raised slightly above the horizontal as in the action of drinking.

Food Summaries, Greater Scaup Duck

In the coast region 13 specimens were taken on fresh water and 21 specimens from a salt water habitat; the food is summarized under these headings. The number of specimens examined is indicated by the figure following each of the months represented.

Fresh water habitat—Fraser River, January, 1; March, 1: Cowichan River, December, 3; January, 3; March, 1: Chemainus River, November, 2; December, 1: Lake, Pender Island, March, 1.

Salmon eggs. Two specimens taken in November from the Chemainus river contained salmon egg cases; this was the only food item in one and 75% of the total contents of the other.

Salmon flesh. Broken down salmon flesh was the sole item in one and represented 65 and 95% of the contents of two other stomachs.

Amphipod. Three specimens of *Gammarus limnaeus* represented minor items in a specimen from Pender Island.

Caddis. Encased caddis larvae were the chief item in a December specimen from Chemainus river.

Other insects. More than 100 aquatic dipterous larvae and Tipulidae, made up the chief item in a Pender Island specimen that also contained several Formicidae and Gyrinidae.

Fresh water molluscs. Twenty-five specimens of *Sphaerium* sp. constituted 9% of the contents of a specimen from Pender Island, which also contained *Planorbis* sp. and another gastropod. Fragments of *Pisidium* sp. was the only evidence of food in a Cowichan River specimen.

Marine molluscs. Marine gastropods, remains of an earlier feeding at sea, were present in two stomachs.

Seeds. Seeds of *Carex exsicatta*, and a few seeds of *Rubus* sp., were the only items in one stomach from the Fraser River. Seeds of *Carex* sp. formed the entire contents of one, 40% of a second and 1% of a third, specimen from the Cowichan River. Seeds of *Scirpus americanus* in two stomachs and seeds of *Myriophyllum spicatum* and *Potamogeton* sp. in another were minor items.

Miscellaneous vegetation. Stems of a narrow-leaved grass formed the sole contents of one and 96% of a second specimen from the Cowichan River. Comminuted vegetable matter was the chief item in a specimen from the Fraser River.

Summary.—Thirteen Greater Scaup Ducks taken on fresh water in the coast region had eaten bulrush and sedge seeds and miscellaneous vegetable matter. Salmon eggs, which occurred twice, and salmon flesh, which occurred three times, are more important foods than these analyses indicate. Insects and molluscs were minor items.

Salt water habitat. Gulf of Georgia, January, 2; March, 1: Cowichan Bay, January, 1: Chemainus river mouth, November, 2: Comox, August, 1; March, 2: Departure Bay, January, 3; February, 7; March, 2.

Herring eggs. Opaque herring eggs were the chief item in a March specimen from Departure Bay.

Hydrozoa. The gullet and stomach of a specimen from Departure Bay were filled with a mass of hydroid colonies.

Crustaceans. A small kelp crab, *Pugettia producta* (Randall) and one hermit crab, *Pagurus* sp., constituted 8% of the contents of a well filled stomach of a bird from Departure Bay. *Hemigrapsus* sp. and undetermined crustacean fragments were the chief items in another and a third stomach contained one amphipod.

Molluscs. Gastropods occurred in 17, blue mussel in 6, limpets in 4, and barnacle scutes in 6 of the 21 stomachs examined. *Littorina* was the exclusive item in two, and predominated in three, other well filled stomachs. In one, the number was estimated to be 800 and in another 400 small specimens. Molluscs, chiefly gastropods, were the only item in six, and represented 70% of the contents of one other, stomach. Species identified: *Alectrion mendicus* Ild., *Columbella gausapata* Ild., *C. permodesta* Dall, *Bittium eschrichtti* (Medd.), *Littorina scutulata* Ild., *L. sitchana* Phillippe, *Lacuna divaricata* (Fab.), *L. variegata* (Carp.), *Acmaea scutum* (Esch.), *A. pelta* (Esch.), *Margarites pupilla* (Ild.), *M. lirulata* (Carp.).

Eel grass. A small amount of eel grass, *Zostera marina*, was present in one stomach and probably had been taken with herring eggs which were also present.

Marine algae. Sea lettuce occurred seven times with an average percentage volume of 28.1. In one specimen it was the exclusive item. An unidentified alga constituted 20% of the contents of another stomach.

Summary.—On salt water by far the most important food is molluscs; the largest whole specimen found (*Alectrion mendicus*) measured 12 mm. Sea lettuce is the food of second, and crustaceans of third, importance. Herring

eggs were noted only once and undoubtedly this was owing to the fact that only one scaup was collected on the spawning grounds.

In Table I is given a food summary of the Greater Scaup Duck in the Okanagan and coast regions of British Columbia.

Economic Relations

In the interior of British Columbia the Greater Scaup Duck is a species of some importance from the standpoint of wildfowling. It is relatively abundant, it decoys well, affords flight shooting in some localities, and it is an excellent table bird. Its food habits would seem to be related to other interests only to the limited extent to which it consumes aquatic insects and is a food competitor of trout or other commercially valuable fishes.

On the coast region its life history for a short time is involved with that of the Pacific herring and with the various species of salmon. So far as known this relationship works only in the interests of the ducks that feed upon the eggs of all these fishes. Any considerable reduction of this food supply might eventually reduce the number of ducks. To what extent, if any, the egg-eating habit of the species adversely affects the propagation of salmon and herring is not known. Most of the salmon ova eaten are drifting eggs of which an unknown percentage is considered to be infertile; the herring eggs taken as food are those that are unattached and of which a large proportion is washed ashore and becomes a waste product. This is discussed in some detail by Munro and Clemens (12, 13) and Munro (10).

The Greater Scaup Duck is not sought by hunters on the coast region chiefly because its flesh is generally considered unpalatable.

Lesser Scaup Duck

Distribution on the Pacific Coast

Summer

The breeding range of the Lesser Scaup Duck in British Columbia has not been mapped in detail. In the southern half of the province it is included in the region lying east of the Cascade Mountains, west of longitude 120° W. and north of latitude 50°. It is a scarce breeder in the Okanagan Valley, which is the extreme southern limit of its breeding range in British Columbia west of the Rocky Mountains, more plentiful in the Kamloops and Nicola regions, and abundant in the Cariboo district. Information is lacking concerning nesting populations in the northern part of the province except in the Atlin district where it has been recorded as a common summer visitant by Swarth (16).

The Lesser Scaup Duck is known to nest commonly in that part of the province west of the Rocky Mountains known as the Peace River district (2). This region is associated geographically with the prairie country and its lesser scaup population may be isolated from any other within the political boundaries of the province, with the possible exception of the Atlin district where the fauna has marked eastern affinities

TABLE I

FOOD OF GREATER SCAUP DUCK, AVERAGE PERCENTAGE VOLUME

Locality	Number of specimens	Salmon eggs	Salmon flesh	Herring eggs	Unidentified fishes	Crustaceans	Insects	Molluscs	Hydroids	*Chara*	Marine algae	Miscellaneous vegetation	Miscellaneous seeds
Okanagan Lake	68				0.03	0.01	1.65	6.08		72.00		19.65	0.58
Swan Lake	11						13.05	.36				16.50	70.09
Fraser River	2											45.00	55.00
Cowichan River	7		36.42					14.85				28.00	20.73
Chemainus River	3	56.67					32.67	9.00					1.66
Lake, Pender Island	1					1.00	62.00	30.00					7.00
Gulf of Georgia	3							100.00					
Cowichan Bay	1							100.00					
Chemainus River (mouth)	2					3.00		97.00					
Comox	3					33.00	.33	53.34			13.33		
Departure Bay	12			4.17		.67		60.58	8.17		23.50	2.91	

North of British Columbia it has been found nesting in southeastern Alaska (3), in east central Alaska (14), in the Yukon Territory (1). In none of these localities is it reported to be common.

So far as known it has not been recorded as nesting south of British Columbia in the State of Washington; there is one record for eastern Oregon and it is reported to nest abundantly in western Montana*.

Winter

In collections of scaup ducks from British Columbia, few winter taken specimens of *N. affinis* are represented. Thus of 54 specimens taken in January and February at Okanagan Landing, only three were of this species (January, 1931) and, of 32 collected in these months at various coast points, two were *N. affinis*. Evidence of the comparative scarcity of the species in winter is borne out by field observations. There have been many opportunities to examine flocks of scaup ducks at close range and none have been identified as the small species. It seems fairly well established that the Lesser Scaup Duck is a scarce winter visitant in British Columbia.

In Washington state it is reported† to be much less common than *N. marila* and restricted almost entirely to a fresh water habitat; no reference is made to its status in winter.

In the Portland region, Oregon, it is the most abundant of the wintering diving ducks, (6), and at Netarts Bay in December, 1912, and January, 1913, outnumbered all other ducks (5). It is said to be the commonest duck during the winter on salt water bays and marshes in California (4).

It is probable that the Oregon and California winter populations are much larger than the total summer populations of British Columbia and include migrants from various widely separated nesting grounds, including some lying east of the Rocky Mountains.

Seasonal Movements

Spring Migration

By what route the summer population of lesser scaups that presumably have wintered in Oregon and California reach British Columbia has not been determined; as the species becomes common in the lower Fraser Valley in late March or early April, it seems probable that one flight takes place along the coast. Thus near Chilliwack on March 29, 1934, flocks with a total of 300 birds were present on sloughs and backwaters of the Fraser River. From this point they probably travel northeast following the general direction of the Fraser and Thompson Rivers. At any rate they appear in the Kamloops and Nicola regions early in April and a migration continues thereafter for a month or six weeks. The time of their greatest abundance is the latter part of April, then flocks congregate on certain lakes where amphipods are plentiful. For example, on Napier Lake, in the Nicola district the following

* *Letter, Bureau of Biological Survey, Washington, D.C., March 6, 1940.*

† *Pp. 797–798 in Birds of Washington, Vol. 2, by Dawson, W. N. and Bowles, J. H. The Occidental Publishing Company, Seattle, Washington. 1909.*

counts were made: 160 on April 25, 1934; 385 on April 27, 1938; 175 on April 25, 1939; 271 on April 22, 1940. The total on 12 small ponds in that vicinity on April 25, 1939, was 465.

At the same time a migration, thought to be composed of birds that had wintered on the lower Columbia River, was following the Okanagan Valley: Numbers congregated at several points, for example, in the Glenmore district, near Kelowna, and on Goose Lake, near Vernon, where the following counts were made: 271 on May 8, 1937; 250 on April 23, 1938; 70 on Goose Lake, April 21, 1939.

On the Arrow Lakes, Kootenay district, the species has been recorded between April 12 and June 3, and is reported to be common (7). May 3, 1933, is the earliest date of arrival at Atlin as recorded by Swarth (16). The numbers noted above are much smaller than those recorded for the Greater Scaup Duck on its spring migration and a comparison reflects the numerical status of the two species in British Columbia.

Autumn Migration

The return flight in autumn appears to follow the same course as that taken in spring. An exodus of adult males and yearlings of both sexes from the nesting grounds takes place soon after the flight feathers have been renewed in August. Very likely these birds make a long journey at the outset; at any rate, few are seen at this time in the Okanagan, which is close to the southern part of the principal nesting ground, or in the lower Fraser Valley. It is of interest to note that migrants have been observed in the Portland, Oregon, district as early as August 10, although the majority do not arrive there until mid-September (6).

In September, there is a second flight from the nesting grounds, of some part of the populations that have been disturbed by gunners. Finally, in October and early November, the third and largest movement takes place. Dates of their last appearance in the Cariboo region are not available but it seems probable that a few remain until the freezing of the small lakes, which normally takes place in early November. The latest record of appearance at Atlin is October 17, 1931, (16).

No information is available concerning a possible migration along the coast line north of the Fraser River. Kelso (7) refers to the species as common on the Arrow Lakes (August 13 to November 8) presumably the earliest and latest date on which individuals have been seen.

Three returns from a total of 29 Lesser Scaup Ducks banded at Buffalo Lake, Cariboo, British Columbia, are of some interest. The details are:

Banded	*Recovered*
Sept. 18, 1932.	Deer Lake, Ore., Nov. 9, 1932.
Sept. 14, 1933.	Fox Lake, Wisc., Oct. 27, 1933.
Oct. 2, 1933.	Odessa, Wash., Nov. 18, 1933.

Reproduction

Arrival on Nesting Ground and Courtship

The Lesser Scaup Ducks become established on their nesting grounds in the Cariboo region during late April or early May, depending on the time the lakes become free of ice. At the same time, flocks of migrants may be present in other districts to the south. Thus on April 22, 1940, totals of 210 males and 60 females were counted on Napier Lake in the Nicola region. These were in four flocks and in each the members were widely scattered with few associated in pairs. This was a concentration of migrants on a rich feeding ground. On the other hand, a total of 174 on 105 Mile Lake, over 100 miles to the north, April 24, 1940, represented a breeding population plus the usual excess of non-breeding birds. Many of the 39 females present were paired and for the most part the mated birds kept apart from the numerous small groups that were scattered here and there over an area approximately one-half by one-quarter mile. Some of the small groups were thought to be composed chiefly of yearlings, others consisted of one or two females attended by a larger number of males and among these an occasional exhibition of courtship activity was observed. The groups of yearlings sometimes joined together, forming flocks up to 30 or so; these birds were restless and took flight at the least alarm, whereas the paired or courting birds showed less nervousness when approached.

On the same lake on April 27, 1939, the behaviour exhibited by a group consisting of one mated pair and two males was as follows: the mated pair swam slowly side by side with only a few inches separating them; the two other males followed several yards behind. Occasionally the mated male advanced in front of the female for two or three feet, turned and faced her for a moment, then resumed his position by her side. At other times he advanced only a few inches and crowded against her sufficiently to impel her to change her course. At such times she would turn about and the male would immediately press close to her again. Once one of the following males approached the female and she splashed over the water towards him which caused him to retreat.

At Storage Lake on May 10, 1939, a group of eight males and three females were in active motion on the water. The males encircled the females and swam towards them with heads retracted and breasts thrust out—they seemed actually to dance. Then several of the group would dive and the circle would be broken. Finally all would rise and fly swiftly about the lake, now high in the air now low over the surface, then splash into the water where they would reassemble in a circle and repeat the dancing courtship.

While the reproductive process is at full tide the males attend the females constantly and seldom leave them except when the latter are on the nest. When the female of a pair was shot and dropped to the water, the male immediately alighted beside it and for a few minutes, standing almost upright, splashed around the dead bird. Although previously wary it did

not fly until approached within 20 yards. When the male of a mated pair was shot the female flew straight away.

Nesting Grounds

Usually the nesting grounds centre about a lake of moderate depth that has bulrush growth on the shore and adjacent brushy coves. Those that contain an abundance of amphipods and aquatic insect larvae support the largest populations. The small grassy type of slough used by some surface-feeding ducks and the open alkaline sloughs and deep lakes frequented by Barrow's Golden-eye, *Glaucionetta islandica*, seldom attract the Lesser Scaup Duck. The two lakes described below are typical nesting habitats.

Lily Pad Lake is one and one-half miles long by 200 yards wide and is used as an irrigation reservoir, the water level being maintained by a dam across its outlet at the north end. The lake bottom is hard, the shores stony, and the peat-stained water deep to the shore line growth of dwarf birch, willow, and black spruce. These low-lying brush-covered areas adjacent to the lake are flooded during the summer thus providing additional cover for waterfowl; farther back from the lake lodge pole pine and poplar predominate.

The dominant vegetation in the main body of the lake is Yellow Pond-lily, *Nuphar*, and the surface is covered with the leaves of this plant. At the south end is an open bulrush marsh and at the north end a 50 acre marsh of the same type with several deep channels through it, terminating in a *Carex* meadow. Aquatic plant growth, other than *Nuphar*, reaches the maximum of abundance in these marsh areas and includes *Myriophyllum*, *Potamogeton pectinatus*, *P. natans*, *P. pusillus*, and *Polygonum amphibium*. Probably the most abundant vegetable food is the seed of *Scirpus* that is not available in abundance until the crop matures in late summer. The most plentiful animal food is an amphipod; leeches and snails (*Planorbis* and *Lymnaea*) also are numerous.

Tatton Lake is a valuable waterfowl nesting ground divided into three relatively deep areas separated by shallows grown over with heavy bulrush marsh. The entire length is approximately one and one-half miles and the maximum width three-eighths of a mile. The lake is entirely surrounded by open bulrush growth and in August the waters on the outside of this shore-line marsh are covered with filamentous algae in a matted deposit of varying thickness and width through which young ducks have difficulty in moving. On the bottom are dense *Chara* and *Nitella* meadows and this is the dominant growth; next in abundance is *Myriophyllum*. The combination of *Chara* and bulrush seems to be one well suited to a dense population of certain diving ducks and coots, the *Chara* providing an abundance of food in itself and in the various organisms it harbours, the bulrush providing adequate nesting cover.

Nesting

Egg laying probably begins in early June but the majority of nests examined have been found in July and August. The earliest date on which a female

PLATE I

FIG. 1. *Lesser Scaup Duck swimming in front of nest site, Lily Pad Lake, British Columbia.*

FIG. 2. *Lesser Scaup Duck taking flight, Tatlon Lake, British Columbia.*
FIG. 3. *Nest and 15 eggs of Lesser Scaup Duck, 105 Mile Lake, British Columbia.*

was flushed from a nest is July 6 (Lily Pad Lake, 1938), the latest August 10 (Tunkwa Lake, 1939).

A variety of nesting sites are used, the most common being a dry situation under cover of one kind or another and within a few yards of a lake shore. Less frequently a wet, marshy site is chosen. The following sites, except that on the muskrat house, can be considered typical.

Lily Pad Lake—July 6, 1938. Three nests containing eggs ($\frac{1}{6}$ $\frac{1}{7}$ $\frac{1}{13}$) on a narrow, stony peninsula covered with grass, one hidden in a pile of old boughs, another in a grass clump beside a large boulder, the third flush with the ground in an open situation and screened by grasses and *Potentilla*.

Tatton Lake—July 22, 1939. A nest of dry bulrush, 11 in. in diameter and three inches in height, on top of a flattened muskrat house in open bulrush marsh, contained seven eggs; no down had been added.

Watson Lake—July 24, 1939. A nest of dry and green bulrush, the top 10 in. above water in a thick clump of bulrush, contained seven eggs deeply imbedded in the nest material; no down had been added.

Tunkwa Lake—August 10, 1939. Each of four nests on a dry grassy island, 10 to 20 ft. from the water, contained seven eggs all well banked with down.

105 Mile Lake—August 10, 1937. A nest on a small rocky island under thick cover of Loco weed, *Astragalus canadensis*, contained 15 eggs almost completely covered by a mixture of down and rubbish (Fig. 3).

In some cases females when flushed from a nest fly off and do not return to the vicinity until the observer has left, in others they may alight on the water close by the nest and swim back and forth in obvious concern. The latter behaviour perhaps is exhibited chiefly by individuals in the early stages of the reproductive process. An extreme example of this in which a female walked off her nest then returned and defended it by grasping with her bill the fingers of an observer has been recorded by Munro (9). Almost invariably the female when flushed from the nest discharges excrement on the eggs. The voided matter carries a strong odour and the action probably has some protective value.

Behaviour of Females and Young

The majority of the young appear from July 15 to August 10; the earliest date recorded for first appearance of a female with downy young is July 4 (Lily Pad Lake, 1938) and the latest August 23 (Tatton Lake, 1939). In both cases the young were a day or so old. Late broods of young with flight feathers ensheathed have been observed in late September, for example, at Fawn Lake on September 26, 1940.

On lakes containing large populations there is much mixing of broods and banding together of several families. Thus at Watson Meadow slough, July 20, 1937, seven females and 64 young birds of various ages were associated in one flock. The young seemed to attach themselves to the nearest female and during the hour these birds were under observation several changes in

the association of young with different females took place. At one time the population was divided as follows: broods of 8, 9, 15, each led by a female; three females with 25 young; 4, 2, and 1 young birds unaccompanied by females.

The mingling of broods and their joint care by several females is of regular occurrence; for example, at Straight Lake on July 15, 1938, three females accompanied a raft of 34 downy young, and on Tatton Lake, July 21, 1938, three females accompanied 35 downy young. In both instances, the young swam in grouped formation, the females sometimes ahead, sometimes a little to one side. At 150 Mile Lake, July 22, 1938, combined broods totalling in one case 22 and in the other 18 downy young were each accompanied by two females. At the same place on July 17, 1939, a band of 55 downy young was attended by three females, one leading the band and two following in the rear or at the side. The young at times swam in a long line, some singly, others two or three abreast; again they would crowd together in a compact group. Many other instances of communal care of young could be cited.

Upon the appearance of a canoe on their territories, females exhibit, in various degrees, concern for the safety of young. The most common reaction to alarm is for the female to swim rapidly towards the canoe, sometimes flat on the surface, again on her side so that the white underparts are exposed. She surges across the surface in one direction then in another and threshes the water with her wings. Sometimes this is accompanied by a soft, purring note.

When two or more females with combined broods are alarmed, it is usual for one to demonstrate in this manner whereas the other female, or females, leads the broods to another part of the lake or into marsh coves. This behaviour has been observed many times. The demonstration on the water may last for only a few minutes or it may be continued at intervals for a much longer time. Thus at Cummings Lake, August 10, 1940, the behaviour of two females with a combined brood of 17 was as follows: one female led the young, which were alarmed and scampered over the water, into a narrow marsh channel where they disappeared from view; the second female rushed across the water towards the stationary canoe and continued to swim back and forth a few yards from it. When the canoe was turned about and paddled in a direction opposite to that taken by the young, she preceded it for a quarter of a mile in a series of short flights. At the end of each flight she splashed across the water near the canoe in the usual manner.

In the early part of the season, defence behaviour follows this general pattern very closely. One of the several exceptions noted was observed at Abel Lake, July 23, 1918, where two females that accompanied a total of 25 young did not react in this way. Both left the young, which were travelling in single file along the outer edge of the Yellow Pond-lily growth that encircles the lake, alighted on the water 50 yards or so in advance of them and, after swimming a few yards, again took to wing and left the vicinity. Meanwhile the combined broods, still in single file, continued on their course for 20 yards

or so, then turned at right angles and scampered over the lily-pads, swimming and running, to disappear in the thick *Scirpus* growth. At no time did the adult females exhibit any of the usual maternal reactions to alarm.

Maternal care becomes less marked later in the summer. Thus at Brigade Lake (August 7, 1939) only two of the six females present, with broods, reacted to alarm in the familiar manner. So also at 105 Mile Lake on August 22, 1939, females showed less concern for their young than had other females under similar circumstances three weeks earlier. On the latter date, each of eight broods, with a total of 58 birds ranging from downy young a few days old (two broods) to one brood about one-third grown, was accompanied by a single female. All but one of the females flew to another part of the lake on being disturbed. When left thus these young either ran over the water or dived, whereas young defended by the female usually swim off with little manifestation of alarm.

In general, it may be said that as the summer advances females exhibit more concern for their own safety and less for that of their young in accord with changes in metabolism as the reproductive process nears its seasonal conclusion.

Mortality of Young

The Lesser Scaup Duck because of its late breeding, when heavy cover is available to shield the nests, appears to suffer less from attack by predators than do some other ducks. The number of young surviving to late summer is higher than with other species and it is not uncommon to find a well grown brood in late August numbering 10 or more.

A primary factor in the relative safety of adolescents is their habit of mingling with bands of moulting adults and yearlings. The older birds are exceedingly wary and when alarmed their behaviour follows a consistent pattern of high protective value as will be explained in the following section. The young are quickly influenced by the actions of the older birds and their reactions to alarm are similar. Another factor of survival value is the generally protective attitude of the female towards the young.

Possible destruction by coots, or by loons and Holboell's Grebe has been discussed by Munro (10). Such loss as may be caused through attack by these birds does not seem to be a factor of importance.

Evidence that Horned owls, *Bubo virginianus*, kill females on the nest has been obtained but such instances appear to be uncommon.

Perhaps one of the chief losses is through drowning or suffocation brought about when the small young become entangled in weeds or matted deposits of filamentous algae, which are abundant on some of the lakes they inhabit. In most cases of this kind observed, it was considered that the small birds had been washed into the heavy growth during rough weather or had dived through an opening in the blanket of algae and been unable to emerge.

Summer Populations

The continued association of lesser scaups in flocks for some time after their arrival on the nesting grounds has been referred to. The size of these flocks begins to decrease in early June as mated pairs move out to nest elsewhere in the general vicinity and for a time the residue may be chiefly yearlings of both sexes, none of which are in breeding condition. A few paired adults sometimes are present as late as July. For example, at Tatton Lake (July 5, 1938) a total of 55 consisted of 10 mated pairs and 35 yearling males. All the mated birds finally disappear and shortly afterwards the population increases again with the return of the first post-breeding males. The number of the latter grows almost daily and in early August adolescent birds that have been reared on the lake also join the company. Thus a favoured lake may finally harbour a large proportion of the local population. At this time the adult males and yearlings of both sexes are moulting their flight feathers and when the moult is completed many move elsewhere, so that constant changes take place in the size of the population. This may be illustrated by an account of conditions at 105 Mile Lake, which is small enough that enumerations are comparatively easy.

On July 13, 1939, this population was composed of 200 adult and yearling males, and 12 yearling females; on July 24, the number had increased to an estimated 260 of which about 40 were flightless; on August 22, the raft consisted of 50 adult and yearling males, 70 adult and yearling females and 50 well grown young. About 20 adults were in flying condition. Elsewhere on the lake on July 24, and later, were females with young broods that kept apart from the older age groups.

Similar observations have been made elsewhere numerous times. Thus at Tatton Lake on August 23, 1939, a smaller association, consisting of 60 males, 40 females, and 70 well grown young, was studied. Most of the old birds were flightless; the largest young had flight feathers ensheathed. There was no difference in behaviour as between flightless adults and young except that the latter were less wary. When disturbed all dived and swam under water to reassemble on a distant part of the lake. Few adults appeared above the surface after the initial dive. Those that did were visible only for an instant before they again dived, sometimes sending a jet of water two or three feet in the air at the moment they disappeared.

Again at Minnie Lake (August 10, 1939) approximately 500 lesser scaups, chiefly adult males and yearlings of both sexes, formed part of a raft which included other species of diving ducks. These birds were exceedingly restless; flightless birds swam long distances under water, and those capable of flight took wing, at the slightest alarm. Thus on this lake, which is a comparatively large one, it was not possible to make satisfactory counts or to determine the various age groups.

Table II sets forth a number of typical summer populations, some restricted to females with young, others including non-breeding yearlings and post-breeding males.

TABLE II

SUMMER POPULATIONS, LESSER SCAUP DUCK

Locality	Date	Adults, ♀, with brood	Young	Adults, ♂, yearlings, ♂	Adults, ♀, yearlings, ♀
150 Mile Lake, 25 acres	July 31, 36	7	70	0	0
150 Mile Lake, 25 acres	Aug. 2, 37	5	57	10	27
150 Mile Lake, 25 acres	July 22, 38	9	85	17	7
150 Mile Lake, 25 acres	July 17, 39	3	55	7	0
150 Mile Lake, 25 acres	Aug. 10, 40	6	64	0	0
149 Mile Lake, 10 acres	July 31, 36	7	64	0	0
149 Mile Lake, 10 acres	July 12, 38	0	0	25	0
105 Mile Lake, 1¼ × ½ mi.	July 31, 36	8	63	175	25
105 Mile Lake, 1¼ × ½ mi.	June 4, 37	0	0	6 (Adult)	6 (Adult)
105 Mile Lake, 1¼ × ½ mi.	Aug. 6, 37	1	13	375	300
105 Mile Lake, 1¼ × ½ mi.	July 3, 38	0	0	210	40
105 Mile Lake, 1¼ × ½ mi.	July 26, 38	5	45	70	30
105 Mile Lake, 1¼ × ½ mi.	Aug. 6, 38	7	60	90	43
105 Mile Lake, 1¼ × ½ mi.	Aug. 16, 38	8	65	140	60
105 Mile Lake, 1¼ × ½ mi.	April 25, 39	0	0	120	80
105 Mile Lake, 1¼ × ½ mi.	July 13, 39	0	0	200	12
105 Mile Lake, 1¼ × ½ mi.	July 24, 39	3	41	210	50
105 Mile Lake, 1¼ × ½ mi.	Aug. 22, 39	11	108	50	70
Watson Meadow Lake, 75 acres	July 20, 37	7	64	0	0
Straight Lake, 1⅜ × ⅛ mi.	July 15, 38	7	78	0	0
Brigade Lake, 1 × ⅛ mi.	Aug. 7, 39	6	72	100	20
Tatton Lake, 1 × ¼ mi.	July 22, 39	3	17	60	12
Tatton Lake, 1 × ¼ mi.	Aug. 23, 39	15	111	60	28
Lily Pad Lake, 1½ × ¼ mi.	July 21, 39	4	20	50	14
Swan Lake, Okanagan	July 31, 40	2	16	0	0

Moults and Plumages

Interest in the plumage sequence of the Lesser Scaup Duck is stimulated by study of the summer populations, where a wide variety of plumage comes under observation.

When the flocks first arrive on the nesting grounds the males of the previous year may sometimes be distinguished from adult males by their generally darker appearance owing to the admixture of grey on the white areas. This distinction becomes more pronounced in early July when young males are rapidly going into an eclipse and the adult males are still in breeding dress (Fig. 4). For example, it was noted at Tatton Lake on July 8 that in a population consisting of 10 mated pairs and 35 yearling males, the former could readily be distinguished by their conspicuous white flanks. Later as adult males eclipse, this distinction disappears and, in August, with occasional exceptions, all look alike.

In the spring, the distinctive white areas at the base of the bill on the female is apparent in most individuals and in the field those of the previous years cannot readily be distinguished from adults. Later the rich brown of the breeding female wears to a lighter, more golden, shade; in some the white

area at the base of the bill is entirely obscured; in others it is retained and in some a second white patch appears on the cheek (Fig. 5). In June most of the incubating females lack the white areas on the face. Thus at Tatton Lake, June 11, 1940, one in 16 mated females had conspicuous white cheek patches.

Meanwhile a rapid fading is taking place in the plumage of the yearling females so that by July these non-breeding birds are much lighter in appearance than the adult females with broods. There is no uniformity in the progress of the plumage disintegration and no two individuals appear exactly alike (8). The difference in coloration between adult and yearling females was illustrated at 105 Mile Lake (August 6, 1938), when a flock of seven yearlings and one adult swam past in regular alignment. The yearlings were faded on neck and head to a pale buff or cinnamon whereas these regions on the adult were rich brown in contrast.

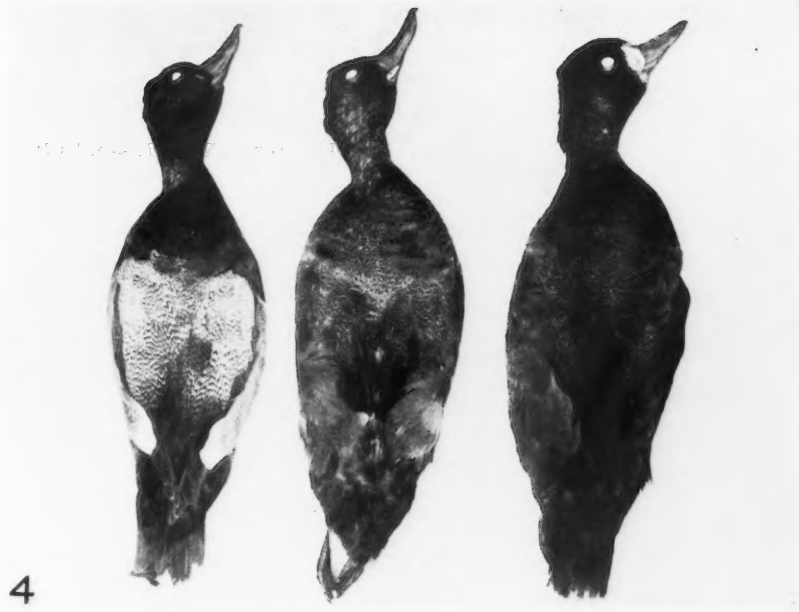
In late August and September young males, as seen under the usual conditions of field study are darker than young females and show more conspicuous white areas about the base of the bill (Fig. 4). This marking is absent in some young females but apparently is always present in the newly moulted adult and yearling females. However, at this time the amount of individual variation among both adults and young is too great always to permit satisfactory field identification of sex and age groups.

Sex Ratio

In flocks of spring migrants that contain adults and yearlings of both sexes there is usually an excess of males over females in the ratio of four or five to one. Examples: Napier Lake, April 22, 1940, 210♂, 61♀; 103 Mile Lake, April 23, 1940, 60♂, 20♀; 105 Mile Lake, April 23, 1940, 135♂, 39♀; Sepa Lake, April 24, 1940, 50♂, 15♀. On the nesting grounds in early summer, where concentrations of non-breeding birds, chiefly yearlings, take place, the excess of males is even greater. At this time the picture is obscured because such flocks may contain some adult males that have bred earlier in the season and may lack a corresponding number of adult females that are incubating eggs or caring for young. Furthermore, the number of yearling females present may represent less than the actual population of this age group; because of the excess of males over females, some yearling females may breed and thus not be present in these associations. However, taking all this into account, there does appear to be a very definite male preponderance in the sex ratio.

Food Studies of Lesser Scaup Duck

The Lesser Scaup Duck of any age appears to obtain most of its food by diving. In general the feeding habits are similar to those of the Greater Scaup Duck except that the former seems more inclined to keep moving fairly rapidly as it feeds. Thus on a narrow, deep lake about a mile in length a flock was seen to fly down-wind and alight near the end of the lake. Here they separated and immediately afterwards started swimming up the lake against the wind. Every few minutes they dived, slipping under water



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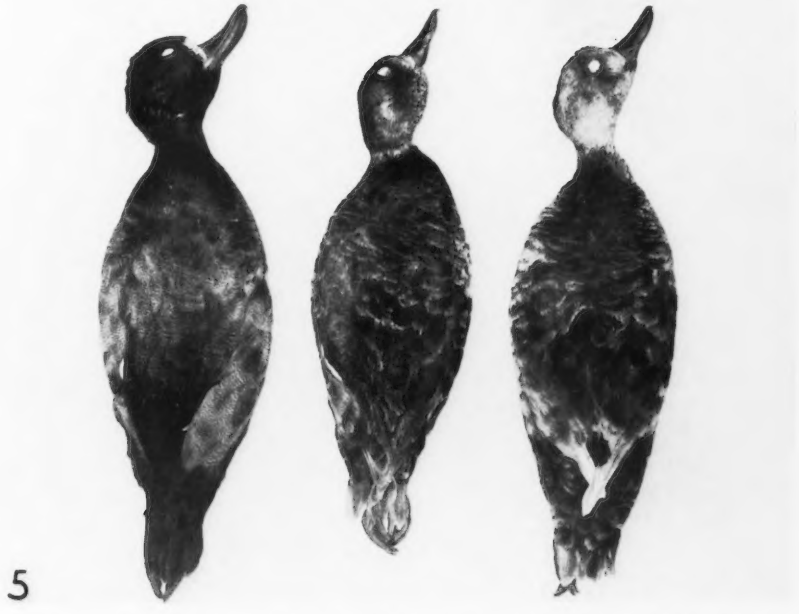

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FIG. 4. *Dorsal view, male Lesser Scaup Ducks; left to right—adult, winter and breeding plumage (June 13); eclipse (August 6); approximately two months old (September 25).*

FIG. 5. *Dorsal view, female Lesser Scaup Ducks; left to right—adult, winter (December 5); adult, breeding (June 13); yearling, showing extent of summer fading (August 22)*

quietly to reappear in from 10 to 20 sec. This manner of feeding and travelling was continued until the ducks had traversed the length of the lake.

The following section summarizes the food eaten by 57 Lesser Scaup Ducks taken in various localities in the interior and on the coast region of British Columbia; the number following each month indicates the number of specimens taken.

Food of downy young—Cariboo region, 150 Mile Lake, July, 1; Watson Meadow Lake, July, 1; 105 Mile Lake, August, 4.

Amphipods. Fragments of amphipods occurred in all three and was the main item in one.

Aquatic insects. A chironomid larva was a minor item in one, Corixids occurred in another, and unidentified insect fragments represented 39% of the food in a third specimen.

Coleoptera. Fragments of a terrestrial beetle constituted the chief item in one stomach.

Molluscs. Shell fragments were present in one specimen.

Seeds. The chief item in one stomach was seeds of *Potamogeton pectinatus*, *Eleocharis palustris*, *Polygonum* sp., *Scirpus* sp.

Food of older young—Cariboo region, Tatton Lake, August, 2; September, 2; 105 Mile Lake, August, 1; September, 4.

Amphipods. Comminuted amphipods formed 80 to 100% of the food remains in seven stomachs.

Aquatic insects. Corixids and fragments of unidentified species were minor items in four specimens.

Hymenoptera. Ants formed one-half the contents of one stomach that was less than one-quarter filled.

Seeds. *Scirpus* seeds were present in four and were the exclusive item in one bird.

Summer food of adults—Cariboo region, Tatton Lake, June, 2; August, 1; 105 Mile Lake, August, 6.

Amphipods. Amphipods were present in the nine specimens and composed 95 to 99% of the contents of seven; in one, approximately 200 specimens of *Gammarus limnaeus* had been eaten.

Aquatic insects. Corixid fragments were minor items in three and *Odonata* nymphs occurred in two specimens.

Coleoptera. Fragments of a terrestrial beetle were present in one stomach.

Seeds. A small number of seeds were present in all but one of the specimens examined, the species represented being *Scirpus* sp., *Myriophyllum spicatum*, *Potamogeton pectinatus*, *Polygonum amphidium*.

Miscellaneous vegetable matter. Unidentified plant material was present in one specimen.

Autumn food—Cariboo region, Disputed Lake, 1; Longbow Lake, 5; 103 Mile Lake, 1; 105 Mile Lake, 2; Fawn Lake, 12 (representing period September 23–October 6, 1940).

Amphipods. Amphipods were found in 14 of the 21 specimens in this group; in one-half this number they represented 90 to 100% of the total stomach contents, in two others 50 and 60%, in the remainder two to five per cent. One specimen contained over 180 specimens of *Hyalella azteca*.

Aquatic insects. In one stomach *Odonata* nymphs constituted the exclusive item and in another represented 75% of the total contents. Corixids occurred as small fragments in seven and caddis in two stomachs.

Molluscs. A single *Planorbis* was present in one specimen.

Miscellaneous seeds. Seeds of *Eleocharis palustris*, *Scirpus* sp., *Potamogeton pectinatus*, *Potamogeton heterophyllus*, *Myriophyllum spicatum*.

Miscellaneous vegetation. This item was third in total percentage volume and consisted chiefly of comminuted vegetation. Several large fragments were identified as stems of *Potamogeton* sp.

Autumn and winter food—Okanagan Region.

Okanagan Lake, January, 3.

Aquatic insects. A caddis larva and fragments of a Corixid were present in one specimen.

Chara. *Chara* was the exclusive item in one well filled stomach.

Miscellaneous vegetable matter. Comminuted vegetable matter was the exclusive item in 1 and 80% of the total in another stomach. *Scirpus* seeds were present in one specimen.

Swan Lake, October, 1; November, 3; December, 4.

Amphipods. Six specimens of *Hyalella azteca* formed a minor item in one stomach.

Aquatic insects. Eighteen damsel fly nymphs and 31 whole Corixids, plus fragments of both, formed the largest item in one well filled stomach. Corixid fragments were present in four other specimens and microcaddis larva cases in two. Chironomid larvae constituted in one specimen 85% and in another four per cent of the total food, the numbers present being 30 and 10 respectively. Two Gyrinid beetles were present in one specimen.

Molluscs. Opercula of small gastropods were present in two and small *Planorbis* in a third stomach, in each case representing a small percentage of the food.

Leech. Leech egg cases were found in one stomach.

Miscellaneous seeds. Seeds of *Ceratophyllum demersum* were the chief item in each of four stomachs that otherwise contained little food. Seeds of *Scirpus americanus* were present in one, *Potamogeton pectinatus* in a second, and *Potamogeton* sp. in each of two other stomachs.

Miscellaneous vegetable matter. Tubers, leaves, and winter buds of *Potamogeton pectinatus*, together with unidentified plant material, composed 39% of the contents of the October taken specimen. Unidentified plant material formed slightly less than one-half of the contents of a second and was the chief item in a third stomach.

Winter food—Coast Region: Fraser River, November, 2; Cowichan Lake, December, 1; Chemainus River, December, 1.

Insects. One *Odonata* nymph was in the Cowichan Lake specimen.

Molluscs. Comminuted mollusc shells were the only evidence of food in the Chemainus River specimen; one specimen of *Planorbis* and one of *Pisidium variable* were in the Cowichan Lake specimen.

Seeds. Seeds of *Potamogeton pectinatus* were present in two, and seeds of *P. heterophyllum* and *P. foliosus* in one, of the Fraser River specimens.

Miscellaneous vegetable matter. Comminuted plant material was the chief item in two specimens from the Fraser River.

Summary.—As may be seen from Table III, amphipods predominated in the food of all age groups from the nesting ground in the Cariboo Region; seeds of aquatic plants were next and aquatic insects third in importance. *Chara* and other plant material were the chief food of three winter taken specimens from Okanagan Lake; plant material and insects predominated in the food of eight late fall and early winter specimens from Swan Lake; on the coast molluscs and vegetable matter were represented in about equal proportions.

TABLE III

FOOD OF LESSER SCAUP DUCK, AVERAGE PERCENTAGE VOLUME

Locality	Number of occurrences	Amphipods	Insects	Molluscs	Leech	Miscellaneous vegetation	Miscellaneous seeds	*Chara*
103 Mile Lake	1	5.00					95.00	
105 Mile Lake	14	62.72	18.35			0.36	18.57	
Tatton Lake	7	72.00	8.28	10.00		.14	9.58	
150 Mile Lake	1	95.00	5.00					
Disputed Lake	1	100.00						
Longbow Lake	5	79.80	20.00				.20	
Fawn Lake	12	26.58	18.67			29.59	25.16	
Watson Meadow Lake	1		39.00	1.00			60.00	
Okanagan Lake	3		6.00			60.00	.67	33.33
Swan Lake	8	0.12	22.00	.63	0.63	22.25	54.37	
Cowichan Lake	1		50.00	30.00			20.00	
Lower Fraser River	2					96.50	3.50	
Chemainus River	1			100.00				

Economic Status

The remarks made with reference to the value of the Greater Scaup Duck as a game bird apply equally to *N. affinis*.

Animal food eaten during the summer on the nesting grounds is composed chiefly of amphipods and aquatic insects, both valuable food for trout, and in some few localities the Lesser Scaup Duck and trout might compete for such food. By far the largest amount of nesting, however, is on shallow, muddy, or alkaline waters that are not inhabited by trout. Consequently such competition as may take place is of little economic importance.

The Lesser Scaup Duck has not been found feeding on the eggs of either salmon or herring but it seems likely that some of the scaups that visit salmon streams in the late fall are of this species.

Acknowledgments

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